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ANIMAL CALORIMETRY: PAPER VIII. THE ALLEGED INFLUENCE OF THE ADRENALS ON DIABETIC METABOLISM *

GRAHAM LUSK
WITH THE ASSISTANCE OF
J. A. RICHE
NEW YORK

The discovery by Blum[1] that administration of epinephrin caused glycosuria at once suggested that its action might be due to an effect on the pancreas. This hypothesis was tested by Herter and Wakeman,[2] who found that direct application of epinephrin solutions to the pancreas caused the appearance of sugar in the urine. Benedicenti[3] and Pemberton and Sweet[4] showed that epinephrin injections diminish the flow of pancreatic juice. The last-named authors suggested the possibility that the "internal secretion" of the pancreas was likewise inhibited and therefore diabetes resulted. Edmunds,[5] however, set forth that the inhibition of the pancreatic flow was due to a reduced blood-supply to the gland brought about by vasoconstriction.

An elaborate theory as to the action of epinephrin was promulgated by Falta, Eppinger and Rüdinger,[6] a theory which has had the support of von Noorden,[7] whose pupils these authors were. According to this theory, epinephrin inhibits the internal secretion of the pancreas, thereby preventing the oxidation of sugar; it stimulates the thyroid to increased

* This article and the six following, viz., those of Graham Lusk and J. A. Riche, E. R. Baldwin, J. A. Hartwell, J. P. Houget and Fenwick Beekman, C. F. Craig, Horst Oertel, C. W. Field, and W. W. Herrick are contributions in commemoration of the hundredth anniversary of the foundation of the medical department of Yale University, and will be republished in book form.

* Submitted for publication March 3, 1914.

* From the Physiological Laboratory, Cornell University Medical College, New York.

1. Blum: Deutsch. Arch. f. klin. Med., 1901, lxxi, 146.
2. Herter and Wakeman: Virchow's Arch. f. path. Anat., 1902, clxix, 479.
3. Benedicenti: Arch. ital. d. biol., 1905, xcv, 1.
4. Pemberton, Ralph, and Sweet, J. E.: The Inhibition of Pancreatic Activity by Extracts of Suprarenal and Pituitary Bodies, THE ARCHIVES INT. MED., 1908, i, 628.
5. Edmunds: Jour. Pharmacol. and Exper. Therap., 1911, ii, 559.
6. Falta, Eppinger and Rüdinger: Ztschr. f. klin. Med., 1908, lxvi, 1.
7. Von Noorden: New Aspects of Diabetes, 1912.

activity, thereby causing an increase in protein metabolism, and it causes a transformation of sugar into fat.

Although not one of these statements is true, the theory has possessed a charm from which many have not been able to free themselves, and it has also been productive of work which has shown the matter in its proper light.

Thus, Ringer[8] rendered dogs diabetic with phlorhizin according to a method originally perfected at the Yale Medical School by Reilly, Nolan and Lusk,[9] and after freeing the dogs of glycogen by shivering, found that administration of epinephrin caused no increase of either sugar or of nitrogen in the urine. Epinephrin, therefore, does not cause the production of sugar from fat nor does it increase protein destruction through thyroid stimulation.

The present paper deals with the remaining question whether or not epinephrin glycosuria is due to an inhibition of the internal secretion of the pancreas, which thereby renders the organism truly diabetic in the sense that there is a decreased power to oxidize glucose.

This problem has been considered by other authors, but their published results are conflicting. Thus Hári[10] administered epinephrin intraperitoneally and intravenously to curarized dogs from twenty-four to thirty-six hours after the last food ingestion, and found that the respiratory quotient was somewhat increased above the level existing prior to the injection of the drug. The increase in the respiratory quotient was usually coincident with a rise in blood-pressure. Wilenko[11] employed rabbits under urethane anesthesia and found that after the ingestion of sugar the respiratory quotients of normal animals ranged between 0.90 and 0.98. Wilenko found, however, that if epinephrin were administered, the respiratory quotient did not rise under the influence of sugar ingestion. From this he drew the conclusion that epinephrin prevents the oxidation of carbohydrate in the organism.

Fuchs and Roth[12] administered epinephrin subcutaneously to three persons, one of whom had Addison's disease, and found an increase in the respiratory quotient. Similar results which were reported by Falta[13] have been described in full by Bernstein.[14]

The question of the inhibition of carbohydrate metabolism through epinephrin seemed to be in an unsettled state and one which might be

8. Ringer: Jour. Exper. Med., 1910, xii, 105.
9. Reilly, Nolan and Lusk: Am. Jour. Physiol., 1898, i, 395.
10. Hári: Biochem. Ztschr., 1911, xxxviii, 23.
11. Wilenko: Biochem. Ztschr., 1912, xlii, 49.
12. Fuchs and Roth: Ztschr. f. exper. Path. u. Therap., 1912, x, 187.
13. Falta: Die Erkrankungen der Blutdrüsen, 1913, p. 428.
14. Bernstein: Ztschr. f. exper. Path. u. Therap., 1914, xv, 86.

determined by experiments with the respiration calorimeter constructed by Dr. H. B. Williams in this laboratory.[15]

It may be added that Allen,[16] using other methods, has reached the conclusion that epinephrin glycosuria is without relation to diabetes.

EXPERIMENTAL

The work was accomplished with two dogs. Both animals were given 0.001 mg. of epinephrin (adrenalin hydrochlorid of Parke, Davis and Co.) per kilogram of bodyweight administered subcutaneously. The

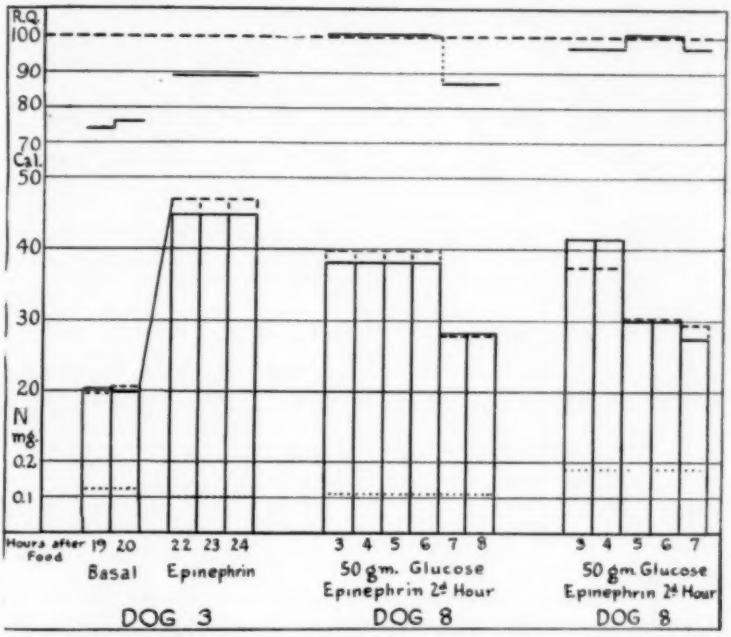

Chart showing the influence of epinephrin on metabolism. Solid lines represent calories calculated, broken lines calories found, and dotted lines milligrams of nitrogen.

animals were brought into the calorimeter as soon after the injection as possible. The urine of the period was collected by catheterization. The dogs were very active while under the influence of the drug, exhibiting unrest and polypnea as has been described by various authors. Dog 3, which had rested quietly in the calorimeter on twenty-one previous occasions, became quite violent after receiving epinephrin, incidentally

15. Williams: Jour. Biol. Chem., 1912, xii, 317.
16. Allen: Glycosuria and Diabetics, 1913, p. 1063.

removing the thermometer from the rectum and chewing it into pieces. The rectal thermometer was therefore not used in the experiments with Dog 8. On this account the direct and indirect measurements of heat are not so close together as they might be. A wire netting was made to surround the dog bed so that the valuable internal adjustments of the calorimeter should not suffer damage.

TABLE 1.—

Date		Time	CO_2 gm.	O_2 gm.	R. Q.	H_2O gm.	Urine N gm.	Non Protein		
								CO_2 gm.	O_2 gm.	R. Q.
3/28/13	Dog 3	a. m. p. m. 11:00-12:00	6.39	6.13	0.76	8.12	0.120	5.27	5.12	0.75
	Exp. 21	p. m. p. m. 12:00- 1:00	6.38	5.94	0.78	7.01	0.120	5.26	4.93	0.78
	Exp. 22	p. m. p. m. 2:00- 5:00	48.13	39.18	0.89	65.95	0.315	45.18	36.52	0.90
4/30/13	Dog 8	a. m. p. m. 11:00- 3:00	61.16	43.39	1.02	103.25	0.428	57.16	39.77	1.04
	Exp. 1	p. m. p. m. 3:00- 5:00	19.67	16.50	0.87	35.74	0.214	17.67	14.69	0.88
5/ 5/13	Exp. 2	a. m. p. m. 11:00- 1:00	31.75	23.92	0.97	51.97	0.354	28.44	20.93	0.99
		p. m. p. m. 1:00- 3:00	24.30	17.18	1.03	45.07	0.354	20.99	14.19	1.08
		p. m. p. m. 3:00- 4:00	10.58	7.90	0.97	20.05	0.177	8.93	6.40	1.01

Necrosis of the skin always follows subcutaneous injections of epinephrin given in these quantities, but no permanent injury ensues. Dog 3 retains a scar on the back at this writing, nearly a year after the treatment, and has experienced fifty experimental periods in the calorimeter.

The full details of all the experiments are given in Table 1, and are graphically illustrated in the accompanying chart.

A. THE EFFECT OF EPINEPHRIN INJECTIONS ON THE BASAL METABOLISM

The *basal metabolism* in this series of papers has been taken as the average heat production during two or three hours of absolute rest following the eighteenth hour after the ingestion of a maintenance ration. Benedict has lately described this metabolism as being "post-

absorptive." Since the basal metabolism or *Grundumsatz* may remain at the same level during months or even years, it seems unnecessary to change its name. It appears to be a desirable expression representing a uniform base line which is immediately affected by changes induced by food ingestion, mechanical work, by variations in external temperature, by drugs or by disease.

DETAILS OF EXPERIMENTS

Calories				Body Temperature			Weight, Mg.	Behavior of Dog	Remarks
Protein	Non Protein	Total Calc.	Total Found	Start	End	Dif.			
3.18	16.99	20.17	19.53	37.68	37.39	—0.29	12.15	Quiet.	Basal metabolism.
3.18	16.48	19.66	20.60	.....	37.58				
						+0.19	.....	Quiet.	
		39.83	40.13						
8.35	125.90	134.25	140.62	38.00	38.83	+0.83	12.15	Very active.	12 mg. epinephrin, 1:13 p. m.
11.52	141.45	152.97	159.32	.....	.....	.....	11.0	Very active.	50 gm. glucose, 9 a. m. 11 mg. epinephrin, 10:15 a. m.
5.76	50.37	56.13	55.56	.....	.....	.....	.....	Active and quiet.	
		209.10	214.88						
9.38	73.76	83.14	74.88	.....	.....	.....	11.0	Very active.	50 gm. glucose, 9 a. m. 11 mg. epinephrin, 10:16 a. m.
9.38	50.71	60.09	60.24	.....	.....	.....	.....	Active and quiet.	
4.69	22.61	27.30	29.51	.....	.....	.....	....	Quiet and movement.	
		170.53	164.63						

The basal metabolism of Dog 3 (weight = 12.1 kg.) was determined between 11 a. m. and 1 p. m., beginning eighteen hours after the ingestion of a standard diet consisting of 100 gm. of biscuit meal, 100 gm. beef heart, 20 gm. lard and 10 gm. of bone ash. This diet contains 688 calories, of which 137 calories (20 per cent.) are in protein, 251 (36 per cent.) in fat and 300 in carbohydrate (44 per cent.) The quantity of carbohydrate in the biscuit meal amounted to 73 gm.[17]

At 1 p. m. the dog was removed from the calorimeter, catheterized, and at 1:13 p. m. received a subcutaneous injection of 12 c.c. 1:1,000 epinephrin solution. The animal was returned to the calorimeter and the experimental work was resumed at 2 p. m. The animal was extremely active throughout the experimental period of three hours. Between 2

17. Lusk: Jour. Biol. Chem., 1912, xiii, 186.

p. m. and 3:52 p. m. (when the rectal thermometer ceased recording) the body temperature of the dog increased 0.83°.

Table 2 shows the influence of epinephrin injection on the basal metabolism of the dog.

This experiment shows that the injection of epinephrin does not increase the nitrogen in the urine. During the period of the basal metabolism, calculations[18] made in the light of the respiratory quotients show that 0.8 gm. of glucose was oxidized per hour, amounting to 16 per cent. of the total heat evolved, while, during the period of epinephrin influence, 7.4 gm. of glucose were oxidized per hour during three hours, yielding 62 per cent. of the total heat produced. The oxidation of sugar was increased ninefold. The results are to be explained by the well-authenticated fact that the administration of epinephrin causes hyperglycemia[19] through discharge of sugar from the glycogen stores of the body.[20] The 22 gm. of glucose oxidized during the three-hour period corresponded to less than 0.2 per cent. of glycogen in the well-nourished dog, a very moderate quantity. The urine of the experimental period was free from sugar, but after the usual standard diet had been administered to the dog following its removal from the calorimeter, the urine became charged with sugar.

The increase of 125 per cent. in the metabolism may be entirely attributed to muscular movements.

This experiment shows that *epinephrin administered twenty-one hours after food ingestion does not prevent the oxidation of glucose in a well-nourished dog.*

B. THE EFFECT OF EPINEPHRIN INJECTIONS ON THE METABOLISM AFTER GLUCOSE INGESTION

Two experiments were performed on Dog 8, a small, fat animal, weighing 11 kg. The standard diet was given daily at 5 o'clock beginning on the evening of April 29. As the dog was not trained to lie quietly in the calorimeter the basal metabolism was not determined. It could scarcely have exceeded 20 calories per hour.

At 9 a. m. 50 gm. of glucose in 150 c.c. of water were given to the dog, and at 10:15 a. m. 11 c.c. epinephrin, 1:1,000, were given subcutaneously; the experiment began at 11 a. m. The results may be condensed as in Table 3.

Experiment 1 shows that the urinary nitrogen was 0.107 gm. per hour, which is not above the usual output of nitrogen during the period

18. For the method of calculation see Williams, Riche and Lusk: Jour. Biol. Chem., 1912, xii, 356.

19. Vosburg and Richards: Am. Jour. Physiol., 1903, ix, 35.

20. Drummond and Noel Paton: Jour. Physiol., 1904, xxxi, 92.

TABLE 2.—Experiments 21 and 22 on Dog 3, Showing the Influence of Epinephrin on the Basal Metabolism

	Time	N. in Urine gm.	R. Q.	Calories				
				Protein	Fat	Carbohydrate	Total Calc.	Total Found
Basal metabolism ...	11 a. m. to 12 m.	0.120	0.76	3.18	14.56	2.42	20.17	19.53
Basal metabolism	12 m. to 1 p. m.	0.120	0.78	3.18	12.36	4.12	19.66	20.60
							39.83	40.13
Average per hour.....	.....	*0.120*	.....	*3.18*	*13.46*	*3.27*	*19.92*	*20.07*
Epinephrin metabolism	2 to 5 p. m.	0.315	0.89	8.35	42.81	83.09	134.25	140.62
Average per hour.....	.....	*0.105*	.....	*2.78*	*14.27*	*27.70*	*44.75*	*46.87*

TABLE 3.—Experiments 1 and 2 on Dog 8, Showing the Influence of Epinephrin During a Period of Glucose Absorption

Time	N. in Urine gm.	R. Q.	Calories				
			Protein	Fat	Carbohydrate	Total Calculated	Total Found
11 a. m. to 3 p. m...	0.428	1.02	11.52		141.45	152.97	159.32
3 to 5 p. m.....	0.214	0.87	5.76	20.55	29.82	56.13	55.56
						209.10	214.88
11 a. m. to 1 p. m...	0.354	0.97	9.38	2.76	71.00	83.14	74.88
1 to 3 p. m.....	0.354	1.03	9.38		50.71	60.09	60.24
3 to 4 p. m.....	0.177	0.97	4.69		22.61	27.30	29.51
						170.53	164.63

of basal metabolism of a dog nourished on the standard diet employed in this laboratory. In Experiment 2 the nitrogen elimination was found to be 0.177 gm. per hour, an abnormally high figure, undoubtedly due to the necrosis of the skin brought about by the previous injection of epinephrin.

In Experiment 1 there was an elimination of 7.9 gm. of glucose in the urine during seven hours following the injection of epinephrin. Deducting this amount from 50 gm. actually ingested, there remain 42.1 gm. available for oxidation. The respiratory quotient of the six hours of the experiment was 0.98. Calculating as before, it appears that 46 gm. of glucose, or 7.7 gm. per hour, were actually oxidized to furnish 171.27 calories liberated from carbohydrate in the organism. This represents 82 per cent. of the total heat production during the period. Since the metabolism experiment did not commence until two hours after the administration of the glucose, it is evident that the carbohydrate oxidized during the six hours of the experimental period counted some glycogen drawn from the body's store of that material.

It may be argued that epinephrin injection might produce an effect on the pancreas which might render it more susceptible if the injection were given a second time. The experiment was, therefore, repeated in all its essentials on the same dog five days later. The dog had received the standard diet at 5 p. m. daily during the interim.

In Experiment 2 there was an elimination during six hours of 11.1 gm. of urinary glucose, leaving available for metabolism 38.9 gm. of 50 gm. ingested. During the experimental period of five hours, the respiratory quotient averaged 0.99. The heat produced from carbohydrate was 144.32 calories, which is the equivalent of 38 gm. of glucose, representing an hourly destruction of 7.6 gm. In this instance, 85 per cent. of the total calories of metabolism were derived from glucose.

Comparing these results with former work[21] accomplished on a normal dog which had received 50 gm. of glucose, it becomes evident that *the injection of epinephrin in the dog has no influence on the metabolism of carbohydrate during the period of glucose absorption.*

The cause of epinephrin glycosuria is best explained by the work of Ritzmann,[22] who found that sugar appeared in the urine only when the dose of the drug was sufficient to cause vasoconstriction. Such a condition may bring about anemia of the tissues, under which circumstance there is always a production of lactic acid, a discharge of glucose from the glycogen repositories, a marked increase in the quantity of blood-sugar and consequent glycosuria. Lactic acid may stimulate the respiratory center, causing polypnea. The oxygen absorption is such, however,

21. Lusk: Jour. Biol. Chem., 1912, xiii, 27.
22. Ritzmann: Arch. f. exper. Path. u. Pharmakol., 1909, lxi, 231.

that anemia of the tissues can only be relative, and is not such as to prevent a large oxidative energy production.

SUMMARY

The subcutaneous injection of epinephrin in a well-nourished dog twenty hours after the ingestion of a standard mixed diet sufficient for maintenance caused an increase of the respiratory quotient from 0.77 to 0.89. From the former quotient it may be calculated that 16 per cent. of the heat production was at the expense of glucose, while from the latter it appears that 62 per cent. had that source. Before the injection of epinephrin, 0.8 gm. glucose was oxidized per hour, and after the injection 7.4 gm. per hour. The dog became muscularly active about half an hour after the administration of the drug.

The subcutaneous injection of epinephrin in a dog which had received 50 gm. of glucose resulted, in one experiment, in a respiratory quotient for a six-hour period of 0.98, and a combustion of glucose amounting to 7.7 gm. per hour, corresponding to 82 per cent. of the heat production of the period. On repetition of the experiment six days later, the respiratory quotient was found to be 0.99 during a period of five hours, and the oxidation of glucose amounted to 7.6 gm. hourly, being the equivalent of 85 per cent. of the total calories produced.

The heat produced from the oxidation of 7.5 gm. of glucose is 28 calories, and the basal metabolisms of the dogs did not exceed 20 calories per hour. This is suggestive that epinephrin does not cause a diminution in the power of the organism to oxidize glucose.

The theory that epinephrin causes a production of sugar from fat, decreases the power of the organism to oxidize glucose through inhibition of pancreatic function, and stimulates the thyroid so that protein metabolism is increased, is untenable in any of its particulars.

Cornell University Medical College, New York City.

EXPERIMENTAL STUDIES ON THE BLOOD-SERUM OF COWS IMMUNIZED AGAINST TUBERCULOSIS

"SENSITIZATION" OF LIVING TUBERCLE BACILLI *

EDWARD R. BALDWIN, M.D.
SARANAC LAKE, N. Y.

The experiments given in detail in this contribution relate to some calves that were immunized in 1902 and 1903 by intravenous inoculations of human tubercle bacilli. The results of various serum reactions, including complement deviation, agglutination and precipitation, were published in 1904.[1] Bactericidal and bacteriolytic tests, which were briefly described at that time,[2] were also performed with culture bacilli.

Nearly all the calves were killed at the conclusion of the first-mentioned experiments. Calf 5 was, however, retained for more than ten years, being repeatedly inoculated with cultures and extracts of tubercle bacilli. These were always of the human type, so that the actual test of immunity-inoculation of bovine bacilli was never made. Nevertheless, from the well-known protection acquired by calves under similar treatment, it is fair to assume that this animal was at least relatively immune.

The further study of immune serums in general, and of this animal especially, appeared of sufficient importance to indicate its retention, as in the course of time additional knowledge of serology was developed.

So far as can be discovered from a search of the literature, no instance is mentioned of an immunized bovine being observed over so long a period. At least, no observations of the blood-serum have extended over such a long time, to the best of our knowledge. It seemed, therefore, a sufficient reason for publishing some of the experiments, although they are chiefly of interest to laboratory workers in tuberculosis.

Some of the experiments have already been reviewed.[3] Of these, many were tests for opsonin or bacteriotropin, but the difficulty of deter-

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* From Saranac Laboratory for the Study of Tuberculosis, Dr. E. L. Trudeau, Director.

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1. Studies from the Saranac Laboratory, Jour. Med. Research, 1904, xii, 215.

2. Studies from the Saranac Laboratory, Jour. Med. Research, 1904, xii, 226.

3. Opsonins in Tuberculosis, Tr. Assn. Am. Phys., 1907, p. 517.

mining the varying content of these substances by a normal standard serum was not overcome, owing to the agglutination. It was shown that a specific bacteriotropin was present, but it could not be measured.

Previous experiments had failed to produce antitoxic serums for tuberculosis, so that no extensive trial was made of the neutralizing effect of the immune cow-serums. Neither was the trial made of serum injections on animals or man with the hope of restraining infection as in the more recent experiments of Neporoschny,[4] Ruppel and Rickman,[5] Calmette and Guérin,[6] Pawlowsky,[7] and Sata.[8] The harmful effects of foreign serums in producing anaphylaxis have long been recognized as a drawback to serotherapy. Notwithstanding this, Neporoschny, Ruppel and Sata asserted that their serums, when injected into guinea-pigs, retarded infection. Calmette and others found similar serums harmful and the disease aggravated.

Our interest was centered in the plan of "sensitizing" living tubercle bacilli with immune serums. It was hoped that by saturating tubercle bacilli or their products with the immune antibody from serums, a better vaccine or tuberculin might be produced. The serum *per se* could thus be washed out but its specific virtues retained, anchored to the bacilli.

Within the past three years such a vaccine, made from B. E. tuberculin sensitized by Ruppel's serum, has been patented in Germany by F. Meyer[9] and has been employed as a mild-reacting tuberculin under the designation "S. B. E." It is said to have diminished toxicity in comparison with plain B. E. Neporoschny also used such a vaccine for immunizing dogs, whose serum in turn was employed in the treatment of tuberculosis.[10] We could not show any lessening of toxic effects of B. E. by our experiments.

As our work was with living, intact bacilli and with cow-serums of lower antibody content than the horse- and mule-serums used by Ruppel, it is not possible to say that our results are contradictory in this respect, though it would so appear in the methods here described.

In the details which follow, some protocols of our first attempts at bacteriolysis are included for the sake of completeness.

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4. Neporoschny, S. D. (quoted by Pawlowsky): Ztschr. f. Tuberk., 1911, xvii, 8.
5. Ruppel, W. G., and Rickman, W.: Ztschr. f. Immunitätsforsch., 1910, vi, 344.
6. Calmette, A., and Guérin, C.: Ann. de l'Inst. Pasteur, 1911, xxv, 629.
7. Pawlowsky, A. D.: Ztschr. f. Tuberk., 1911, xvii, 1.
8. Sata, A.: Ztschr. f. Tuberk., 1911, xviii, 1; 1913, xx, 1.
9. Meyer, F.: Berl. klin. Wchnschr., 1910, No. 20, p. 926.
10. Neporoschny, reviewed by Piel P.: Beitr. z. Klin. d. Tuberk., xxi, 311.

BOVINE IMMUNIZATION; WEAK-VIRULENT HUMAN BACILLI

Bactericidal Influence of Immunized Serum from Heifers Nos. 4, 5 and 6 on Cultures

The following notes were made in 1903 of the first tests made with the serum of No. 5 together with others (Nos. 4 and 6). The animal was then 11 months of age and weighed 240 pounds. Three months previously (Dec. 1, 1902), the first immunizing inoculation of weak-virulent living human tubercle bacilli, culture R1, had been administered. The dose was 0.0015 gm. intravenously. The serum was obtained twenty-four hours after bleeding.

EXPERIMENT 74.—March 19, 1903: Culture on agar of human tubercle bacilli, K1, moist growth, March 8, 1903, emulsified in normal saline, centrifuged one minute, gave a strongly opaque emulsion. Took 0.5 c.c. tubercle-bacilli emulsion, and 1.5 c.c. fresh active serums of No. 2 (normal) and Nos. 4, 5 and 6 (immunized); incubated one hour, centrifuged, decanted serums and planted four tubes each from the sediments.

CULTURE GROWTHS IN EXPERIMENT 74

Date	Serum No. 6	Serum No. 4	Serum No. 5	Serum No. 2	Control
March 24	3 good 1 contam.	1 fair 2 slight 1 neg.	Good on all	2 fair 2 slight	2 good
April 20	4 good	2 good 2 slight	Good on all	Good on all	2 good

Result.—No evidence of bactericidal influence on cultures of tubercle bacillus after treatment by calf-serums.

This experiment was repeated three weeks after the second inoculation of Calves 4, 5 and 6 with 0.025 gm. R1 tubercle bacillus.

EXPERIMENT 96.—April 2, 1903: Culture human tubercle bacilli K1 agar, March 19, 1903. Rubbed in mortar with normal sodium chlorid; centrifuged two minutes; weak emulsion. Serums fresh, active, from bleeding April 1. Took 1 c.c. emulsion: 2 c.c. each, Serums 2, 4, 5 and 6. Incubated two hours; centrifuged: A, sediment transferred to agar plates; B, part of the sediments were treated a second time for nine hours with 2 c.c. of fresh serums; then plated on agar.

April 8: A, good growth on all in microscopic colonies; no contaminations; B, slight growth in Serums 5 and 6.

Result.—No bactericidal influence clearly evident by this method.

Other attempts to demonstrate bactericidal effects on cultures were made with the culture R1 and also with virulent cultures at this time but without success. Contaminations were frequent in some tests; in most the cause of failure was the uncertainty of growth after soaking in the serums. The method was therefore limited to the use of Culture K1, which resembled the avian bacillus, though said to have been the direct descendant of Koch's first culture from human sources.

Animal inoculations of tubercle bacilli treated with Serums 2 and 6 immunized had already failed to reveal any bactericidal effect, consequently no further attempts were made with Serum 5 until repeated injections of tubercle-bacilli products over a long period had been made.

Immunized Cow 5, Injected with Water-Extracts of Tubercle Bacilli

During 1904 and 1905 large quantities of filtered water-extracts of tubercle bacilli were injected into Cow 5, both subcutaneously and intravenously. The amounts varied from 25 c.c. to 175 c.c., and frequent tests of the serum were made for specific agglutinin, precipitin and complement deviation.

The agglutination titer[11] reached only 1 to 100 and 0.025 c.c. deviated complement, but the precipitin titer was low—1 to 4.

EXPERIMENT 237.—Oct. 12, 1905; Bactericidal test by inoculation. Cow 5: weight 850 pounds. Tuberculin test, 0.5 c.c. No reaction.

November 14: Bled 100 c.c. aseptically; defibrinated with iron shavings; centrifuged 20 c.c. for serum.

B :10 c.c. defibrinated blood + 1 c.c. emulsion tubercle bacilli virulent culture human H37, Nov. 7, 1905. Four oese to 4 c.c. normal saline.

S : 5 c.c. serum only + 0.5 c.c. as before.

C : 1.5 c.c. normal saline + 1 c.c. as before.

All incubated seventeen hours; no contaminations; centrifuged; B and S diluted; B to 6 c.c.; S to 4 c.c.

November 15: Inoculated two guinea-pigs intraperitoneally with each culture as follows:

Culture	Amount Each in c.c.	No.	Weight gm.	Duration of Life, Days	Average Days
C	1.25	1	450	39	41½
		2	450	44	....
S	2	3	475	13	14½
		4	445	16	....
B	2	5	425	13	22½
		6	460	22	....

Result.—All the animals died of peritoneal tuberculosis with some generalization. The animals inoculated with blood and tubercle bacilli seemed to have a more rapid infection than the controls.

From this and other experiments we concluded that prolonged exposure of tubercle bacilli to normal (0.8 per cent.) saline solution, even in the absence of light, has a weakening action on the vitality of the tubercle bacillus.

BOVINE IMMUNIZATION WITH VIRULENT HUMAN INOCULATION

The immunization methods hitherto employed appeared to produce little effect on the serum reactions of our bovines. It was at least not evident in agglutinin, precipitin and complement-deviation tests to an

11. With Koch's agglutination emulsion, diluted 1 to 1,000.

extent that was expected. More virulent human bacilli were then tried with the hope of stimulating greater reaction responses to tuberculin injections and presumably bacteriolytic antibodies.

To this end, Cow 5, now being full-grown and in excellent condition, weight 850 pounds, was given, Nov. 14, 1905, 0.020 gm. (moist weight) in 10 c.c. normal saline, virulent human culture H37 broth from Oct. 19, 1905, intravenously in the jugular. The highest temperature recorded in the next twenty-four hours was 102.6 F. No ill effects were noticeable, but the cow was left untreated for nearly a year except for occasional bleeding.

Cow No. 15.—A fresh cow, 2 years old, weight 650 pounds, tuberculin test negative, had also been inoculated Oct. 30, 1905, with (0.02 in 10 c.c. normal saline) virulent human culture H38 from broth Oct. 15, 1905, intravenously in the same way as Cow 5. No reaction occurred from the inoculation until the following day, when the temperature reached 103 F. at 5 p. m. Subsequently for three weeks the temperature varied from 103 to 104 F. at the same hour.

The animal gradually resumed her normal condition, but had evidently foci from the inoculation, though not a progressive infection. After a year she was given a tuberculin test simultaneously with Cow 5, and reacted to 0.5 c.c. O. T. to 41.2 C. (106 F.), indicating the persistence of some unabsorbed tubercles resulting from the inoculation. Two days after the test both Cow 15 and Cow 5 were bled for a preliminary test of the serums as to the bactericidal power.

EXPERIMENT 237 B.—Oct. 24, 1906: Bled Cows 5 and 15 two days after tuberculin test; Cow 15 reacting, Cow 5 not reacting.

October 26: Serums separated. Culture of virulent human tubercle bacilli H38 xx. Small portion rubbed in mortar + 15 c.c. normal saline, centrifuged ten minutes. Supernatant emulsion very weak and contains isolated tubercle bacilli.

A : 0.5 c.c. emulsion + 2 c.c. normal saline.

B : 0.5 c.c. emulsion + 2 c.c. active Serum 5.

C : 0.5 c.c. emulsion + 2 c.c. active Serum 15.

All incubated aseptically twenty-four hours at 37.5 C. (99.5 F.).

October 27: Centrifuged A, B and C; pipetted B and C and discarded serums; added fresh serums, respectively, Serums 5 and 15. Incubated forty-eight hours.

October 29: Repeated and incubated twenty-four hours.

November 1: Removed and pipetted all tubes without shaking them. The sediments were examined microscopically for contaminations and for evidences of lysis in the tubercle bacilli. All tubes uncontaminated; no certain evidence of lysis, although some bacilli were vacuolated.

November 1.: Inoculated all sediments into guinea-pigs, subcutaneously, right groin, as follows:

	Pig	Weight, gm.	November 30 Right Inguinal Nodes
A.....	1	375	Not noticeable.
B.....	2	335	Enlarged to size of wax bean.
C.....	3	310	Enlarged to size of wax bean.

December 7: Pig 1 killed thirty-seventh day. Few small nodules in lungs. No caseation in spleen and glands. Smears from spleen, tubercle bacilli negative.

December 9: Pig 3 (370 gm.) killed thirty-ninth day by tuberculin injection 0.5 c.c. O. T. Enlarged right inguinal nodes with caseation; cold abscess in groin; spleen enlarged and congested along its edge; a few tubercles seen outlined in the liver.

December 10: Pig 2 killed fortieth day; tuberculin injection, B. F.; no reaction to 0.5 c.c. In good condition; weight 370 gm. Enlarged and slightly caseous right inguinal nodes; no abscess; mesenteric nodes enlarged and caseous; spleen enlarged and beginning caseation; numerous small caseated areas in liver; none in lungs.

Result.—Clearly no evidence of bactericidal effect on the part of serums. On the contrary, the control emulsion in normal saline apparently failed to infect the animal, whereas repeated treatment with fresh active serums did not destroy enough bacilli, if any, to prevent infection, although but few bacilli were present.

Immunizing Treatment, Cows 15 and 5†*

Cow 15 was given frequent injections of tubercle bacilli water-extracts subsequent to the preceding experiments. They were injected subcutaneously and intravenously and no fever reactions were noted from 20 c.c. doses.

During the immunization of Cow 15 with water-extracts of tubercle bacilli, there was a different method tried on Cow 5. This animal gave no reaction to 0.5 c.c. O. T., Oct. 22, 1906, a year after the inoculation of virulent human tubercle bacilli. The serum agglutination titer was 1 to 33, practically that of a normal cow. Injections were therefore commenced of a preparation of tubercle bacilli residue left after extraction with water and 10 per cent. sodium chlorid followed by alcohol and ether. This residue was emulsified in normal saline, 1.0 gm. (dry) to 300 c.c., and injected in increasing doses from 1.5 c.c. to 35 c.c. subcutaneously and intravenously. Only slight reactions were produced from 25 c.c. of the emulsion subcutaneously. After three weeks' treatment the agglutinin titer reached 1 to 75. An experiment was then made with the serum in comparison with that of a healthy normal cow, using a very weak emulsion of tubercle bacilli for inoculation.

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* The agglutination titer of Serum 15 never exceeded 1 to 100, nor was any precipitin obtained. The animal was slaughtered December, 1907, and the lungs and other viscera were normal in appearance. The lung-hilus lymph-nodes were also normal except that the largest showed a white cicatrix in its center. No inoculations were made with it so that its tuberculous source could only be surmised.

† In March, 1907, some experiments were undertaken with rabbits inoculated intravenously with virulent human tubercle bacilli treated with Serums 15 and 5, and that of Cow 16 (normal), for a control, together with broth and normal saline controls. After the inoculations the ten rabbits were killed at stated intervals during the succeeding six hours with the object of studying the reaction of the tissues to the "sensitized" bacilli. The pathologic study was never made, but other observations about that time tended to diminish the interest in the result.

EXPERIMENT 237 C.—Dec. 3, 1906: *Guinea-Pig Inoculations.*—Cow 5 bled seven days after an injection of 25 c.c. residue subcutaneously, without fever reaction. Normal Cow 16 bled at the same time,

December 4: Serum agglutination test: Serum 5, 1:75; Serum 16, 1:50.

December 4: Prepared a weak emulsion of virulent human culture H39 from serum slant, Nov. 12, 1906, in normal saline centrifuged. Supernatant emulsion pipetted; tubercle bacilli scarce in smears.

Used 0.5 c.c. emulsion + No. 1 Control, 3 c.c. normal saline.

Used 0.5 c.c. emulsion + No. 2 Control, 3 c.c. Serum of Cow 16 inactivated at 65 C. (1.49 F.).

Used 0.5 c.c. emulsion + No. 3——— 3 c.c. active Cow-Serum 16.

Used 0.5 c.c. emulsion + No. 4——— 3 c.c. active Cow-Serum 5.

All incubated twenty-four hours; centrifuged Nos. 3 and 4.

December 5: Changed the serums for fresh active serums by carefully avoiding sediment in pipetting.

December 6 and 7: Same procedure as followed December 5.

December 8: Centrifuged all tubes; pipetted all except Tube 1. Added 3 c.c. normal salt solution to each tube except Tube 1. When well shaken and stirred, smears were made from each for examination. Tubercle bacilli were seldom found, but no morphologic change was seen. No contaminations noted. The contents of the tubes were then inoculated into twenty guinea-pigs, five to each series. Each tube was filled to 3.5 c.c. and 0.7 c.c. inoculated subcutaneously in the right groin of each animal.

Jan. 13, 1907: Thirty-sixth day: Inguinal nodes examined and palpated.

Series 1: No enlargement.

Series 2: Two show hyperplasia; one doubtful.

Series 3: Three show hyperplasia; two doubtful.

Series 4: All show hyperplasia.

January 15: Thirty-eighth day: One pig in each lot killed by puncture. They revealed progressively more disease from Series 1 to 4. No disease found in Series 1.

January 16: Thirty-ninth day: All the other animals were killed and compared. Series 1 revealed no tuberculous lesions to eye. Small gelatinous or translucent nodules found subpleurally in lungs of Series 3, 4 and 5. Inoculations of sections of lung into two guinea-pigs were negative after thirty-three days (nodules probably lymph-nodes).

Series 2: Pig 1, deep inguinal nodes caseated; iliac nodes slightly diseased, otherwise negative.

Pig 2, deep inguinal, iliac and lumbar nodes caseated; spleen involvement doubtful; lungs have translucent nodules as in Lot 1.

Pig 4, superficial and deep inguinal and iliac nodes caseated; lumbar nodes and spleen slightly diseased.

Pig 5 (pregnant near term), deep inguinal and iliac nodes caseated, lumbar nodes and spleen doubtful.

Series 3: Pig 1 (pregnant near term), inguinal, iliac, lumbar and lung-hilus nodes caseous; nodules beginning in spleen which is slightly enlarged.

Pig 2, same as Pig 1, only more pronounced disease in the spleen.

Pigs 3 and 4 same as 1 and 2, except beginning disease in liver. Series 3 distinctly more diseased than Series 2.

Series 4: Pigs 1, 2, 3 and 5 show marked caseation and hyperplasia of inguinal, iliac and lung-hilus nodes; enlarged and nodular spleens; beginning areas in liver and lobular, translucent areas in lung. All of this series was very uniformly diseased and distinctly advanced beyond the preceding series.

Result.—There was a definitely greater infection in animals of the immunized serum Series 5 than in the controls. A similar difference existed between the animals of the control Serum 16, active and inactive, respectively, while the saline control apparently failed to infect at all, as in previous experiments.

Discussion

It again appeared that our immunized cow-serum was more potent in favoring infection than in restraining it. The conditions of the last experiment were so carefully controlled that the accuracy of the observations was unquestionable. The interpretation of them was more puzzling. Several suggestions were considered:

1. Were the bacilli reproducing in the serums during the four days' successive incubation? This is hardly probable, as the growth in inactivated serum would have been more than in active serum presumably exerting its alexin power.

2. Did not the greater agglutinating action of the immunized cow-serum act to protect the vitality of the bacilli by coating them with agglutinins, and by reason of their clumping, render them more difficult of access to the leukocytes of the guinea-pig than single bacilli? This appeared more plausible but leaves no ground to believe that the serum plays an important part in the bacteriolysis of tubercle bacilli. The inference that cellular elements are more involved in the duty of overcoming the tubercle bacilli was plain. Our preliminary experiments with defibrinated blood (Experiment 237A) did not cover this point satisfactorily. Hence a repetition of the work seemed indicated with more care in securing tubercle bacilli free from clumps.

Guinea-Pig Inoculation with Virulent and Weak Tubercle Bacilli

EXPERIMENT 237 C2.—Feb. 1, 1907: Serum and Leukocytes. Cows 5 and 16 were bled, the former seven days after the last dose of tubercle bacilli residue, 20 c.c. The serum was separated at once from defibrinated blood and used to repeat the previous experiments, very few tubercle bacilli being employed. An emulsion of virulent culture of human tubercle bacilli, H39 serum-slant from Dec. 31, 1906, was prepared and centrifuged twelve minutes. It was diluted and the control examined for clumps before being used. Very few tubercle bacilli (1 or 2) were found in an entire smear and only rarely in clumps. With this very weak emulsion the serums were added as follows:

I

A : 0.5 c.c. control emulsion + 3 c.c. Normal saline.
B : 0.5 c.c. control emulsion + 3 c.c. Serum 5 (active).
C : 0.5 c.c. control emulsion + 3 c.c. Serum 16 (active).
D : 0.5 c.c. control emulsion + 3 c.c. Serum 5 (inactive) at 65 C.
All incubated one hour.

In addition to the preceding, an attempt to increase the virulence of tubercle bacilli was made in the following manner:

An emulsion of weak-virulent human culture R1, serum-slant from Jan. 23, 1907, was prepared in the same way and the following mixtures made:

II

A : 0.5 c.c. emulsion + 3 c.c. Normal saline.
B : 0.5 c.c. emulsion + 3 c.c. Serum 5 (active).

All tubes were incubated four successive days, those containing serums being centrifuged, pipetted and fresh serum added each day. All were centrifuged

February 5 and the serums pipetted. Before dilution smears were made of equal (measured in an opsonic pipet) quantities of the sediments to note any evidence of changes in the number and form of the bacilli.

Careful staining and examination failed to show any marked differences. In some slides no tubercle bacilli were found, in others two or three in an entire slide. Series 2 had ten to fifteen to a slide, the emulsion of R1 being stronger than H39.

To the sediments were added normal saline to make 3.5 c.c. in each tube. Thirty guinea-pigs were inoculated in series of five for each test, 0.7 c.c. being injected into the right groin.

The cows were meanwhile bled again aseptically February 2, directly into the tubercle bacilli emulsion H39 prepared the previous day, as follows:

III

A : 0.5 c.c. emulsion + 5 c.c. normal saline and 1 per cent. sodium citrate solution + 5 c.c. fresh blood from Cow No. 5.

B : 0.5 c.c. emulsion + 5 c.c. normal saline and 1 per cent. sodium citrate solution + 5 c.c. fresh blood from Cow 16.

C : 0.5 c.c. emulsion + 5 c.c. 1 per cent. sodium citrate solution (control).

After mixing and incubating six hours the blood-tubes were centrifuged, pipetted and the leukocyte layers transferred to tubes to which 1 c.c. sodium citrate solution was added to prevent coagulation. These were also incubated four days. Smears made of the final centrifuged cells showed numerous leukocytes, but few phagocyted tubercle bacilli in polynuclear leukocytes. A few extracellular tubercle bacilli but no clumps were noted in casual search in a few fields. There was no apparent contamination. The sediments were then inoculated into six guinea-pigs in series of two for each tube.

Necropsies

February 11, sixth day: *No. 4, Series I D* (Serum 5, inactive) died: Nothing abnormal found. Agar and serum-slant cultures from spleen, negative February 12.

February 12, seventh day: *No. 2, Series III A* (leukocytes) died of septicemia (?). Large brawny infiltration at site of inoculation in right groin; beginning suppuration; spleen large. Smears showed no tubercle bacilli or other organisms in pus from groin.

February 13, eighth day: *No. 4, Series II A:* died of septicemia. Right inguinal nodes normal (smears negative to tubercle bacilli); spleen and liver large and soft; hemorrhagic pericarditis; pleuritis exudativa. Infection probably antedated tubercle bacilli inoculation.

February 20, fifteenth day: Remaining animals were examined and the lymph-nodes palpated. All saline emulsion controls—I A, II A, III A and B were normal. I B, C, D, and II B serum animals showed suspicious beginning hyperplasia.

March 5, twenty-eighth day: All examined and distinct enlargement of regional lymph-nodes found in all serum and leukocyte animals. Controls II A Nos. 1, 2 and 3 were questionable.

March 9, thirty-second day: No. 4, Series I A control died of chronic septicemia; lymph-nodes normal; smears from inguinal and bronchial nodes tubercle bacilli negative; liver large, pale (fatty?); lungs show consolidation of cranial and part of caudal lobes; pregnant (3) near term. (This animal was similar to several other old guinea-pigs that were found to have recovered partially from pneumonic septicemia.)

March 16, thirty-ninth day: Seventeen animals were killed by puncture for comparison. One of Series I A, all of the Series I B, C and D and all of III A, B and C.

The controls in normal saline were all found normal. No tubercle bacilli were present in smears from the inguinal nodes.

The four guinea-pigs Series I B—(Serum 5) were all uniformly diseased with tuberculosis. Large caseous inguinal nodes; beginning caseation in iliac, retro-

peritoneal, bronchial and cervical chains; "hobnail" spleens with slight caseation; liver also slightly caseated; small isolated tubercles in the lungs.

Four of Series I C and three of I D were practically the same as the I B animals, except that the caseation and infiltration of lymph-nodes were less marked. (Pig 4, I D was not recorded.)

The three from Series III A and B (leukocytes) were also practically the same as the serum animals.

March 18, forty-first day: The remaining Series II A (saline control) and II B guinea-pigs were killed. They all showed slightly enlarged inguinal nodes and a few cheesy foci which contained degenerated tubercle bacilli. There were no signs of disease beyond inguinal nodes except that in one the iliac nodes were involved.

Series II B (Serum 5): Well-marked involvement of inguinal and iliac nodes with slight caseation in all; moderately enlarged spleen and occasional caseous foci in livers; lungs normal; smears from inguinal and iliac nodes reveal well-stained tubercle bacilli.

Two fresh guinea-pigs were inoculated from the iliac nodes of No. 4, II B and the spleen of No. 1, II B to note any possible increased virulence from passage after treatment by serum from Cow 5. They were both killed July 12, four months later, but were found normal. One of the first controls, Pig 2, I A (saline emulsion), was also killed at the same time (after five months) and found to have chronic tuberculosis in lymph-nodes, spleen and lungs. The other, Pig 1, I A was lost.

Results.—This experiment confirmed the results of the previous ones as to the favoring effect of immunized cow-serum on the infective power of tubercle bacilli, whether of virulent or low-virulent stocks.

The difference between the effect of active and inactive serums, while less marked than might be expected, was distinct, as was that between the immunized and normal serums. No appreciable effect was produced by the leukocytes that could not be accounted for by the influence of the plasma. There was at least no evidence of bactericidal action. The weakening influence of normal saline was again demonstrated, most of the controls being free from macroscopic disease when killed. No increase of virulence could be demonstrated in the culture R1 in the second passage as a result of serum treatment.

Discussion

The cause of the paradoxical increase of infectivity of the serum-sensitized bacilli was in itself a problem of importance and led us into further experiments to discover the cause, if possible. On the one hand we had found no multiplication of the bacilli *in vitro* and no morphologic changes. On the other hand, we knew that the bacilli were agglutinated by the serum in large clumps. It was conceivable that they were protected, as it were, with a substance which was inimical to their growth only in the body of the homologous animal, namely, the immunized cow. Unfortunately we did not have the facilities for the use of more calves. Hence we could not complete the comparison between inoculations of plain and sensitized bacilli. We therefore tried corneal inoculations in rabbits, hoping by direct inspection to observe the course of infection from day to day and thereby discover the cause of the differences.

Corneal Inoculations in Rabbits

EXPERIMENT 237 E.—March 18, 1907: Bled Cows 5, 15 and 16, seven days after injections of tubercle-bacilli residue into the first two animals.

March 19: Serum separated: Agglutination titer: Serum 5, 1 to 100 +; Serum 15, 1 to 100 ++; Serum 16, 1 to 33 to 50.

An emulsion of virulent human tubercle H38xx, serum-slant from March 7, 1907, good growth, was rubbed in 5 c.c. normal saline, centrifuged ten minutes and filtered twice through sterilized paper. The emulsion was faintly opalescent and when diluted once with equal quantities of saline gave smears with 3 to 5 tubercle bacilli to a field and clumps seldom noted. The following mixtures were made:

A: 0.5 c.c. emulsion tubercle bacilli + 5 c.c. active Cow-Serum 5.
B: 0.5 c.c. emulsion tubercle bacilli + 5 c.c. active Cow-Serum 15.
C: 0.5 c.c. emulsion tubercle bacilli + 5 c.c. active Cow-Serum 16.
D: 0.5 c.c. emulsion tubercle bacilli + 5 c.c. broth, 2.5 per cent. glycerin.
E: 0.5 c.c. emulsion tubercle bacilli + 5 c.c. hydrated chloral 1.5 per cent. solution.
F: 0.5 c.c. emulsion tubercle bacilli + 5 c.c. normal saline solution.

All incubated twenty-four hours at 38 C. (100.4 F.) and centrifuged; pipetted + 1 c.c. H_2O to each tube and transferred to small-pointed tubes.

Again centrifuged and sediment used by smearing it on a corneal knife for inoculation. Three rabbits were used for each series, A, B, C and D. Three were used for E and F in the right and left eyes, respectively. The periphery of the corneae near the nimbus was split under cocain to the width of 2 mm. for the inoculation. One animal of each series was killed after six hours and another after seven days for microscopic study (which was later abandoned for other work).

The eyes of the remaining rabbits were watched daily and showed characteristic differences. A general description will suffice to show them. During the first week the earliest to exhibit beginning disease was Series A (serum from Cow 5). Series B was practically the same, while C, D, E and F were progressively less, or had no signs whatever, as in E and F.

During the second week metastatic tubercles began to show in the corneae of A, and a well-developed focus in B and C as well. In the third week subsidence of the series A, B and C was noted, while a gradual beginning disease was observed for the first time in D, E and F.

April 16, 1907: Forty-seventh day: All killed. Relations remain unchanged. A, B and C have progressed slowly; A shows softening in each focus; B is still quite inflamed; metastases well marked; C is moderately inflamed but less than A and B; D has very slight inflammation remaining; E none; F progressive.

Necropsies revealed no internal disease except a few coccidia in two rabbits.

Result.—A confirmation of previous experiments on guinea-pigs in that the sensitized bacilli showed an accelerated infection.

The slight difference between the course of the disease in A, B and C in the rabbits' corneae raised the question anew whether or not there was any specific influence of the immunized serums on the course of infection. The following experiment was made to test this point in part, though the result was of no great importance.

EXPERIMENT 237 F.—April 3, 1907: *Corneal inoculations in rabbits.*—Five rabbits were each inoculated in the cornea of one eye with twice the dose of twenty-four-hour sensitized bacilli with control serum than were inoculated in the other eye with Serum 5. After thirty days the control eyes showed more disease than the others. So far as this result could be applied to the question, the number of tubercle bacilli required to infect the eyes to an equal degree was less than twice the number treated with the immunized serum.

Cow 5 had no immunizing injections after March 11, 1907, until the following November. During July, four months after the last injection

of tubercle bacilli residue, the serum was again tested in comparison with Control 16 by another experiment on guinea-pigs.

EXPERIMENT 237 G.—July 27, 1907: *Guinea-pig inoculations.* Cows 5 and 16 bled.

July 29: Serum separated and mixed with an emulsion of virulent tubercle bacilli. H39 slant-culture from June 12, 1907, containing four to five single bacilli to a field.

1. Took 0.5 c.c. normal saline emulsion and 3 c.c. active serum 5.
2. Took 0.5 c.c. normal saline emulsion and 3 c.c. active serum 16.
3. Took 0.5 c.c. normal saline emulsion and 3 c.c. inactive serum 16.

All incubated twenty-four hours, then to each tube 10 c.c. normal saline was added and centrifuged without stirring. The sediments were washed twice with 1 c.c. normal saline and finally diluted with 2 c.c. normal saline for inoculation. No contaminations.

Three lots each of eight guinea-pigs, were given 0.25 c.c. in the right groin. They were all killed on the forty-fourth day and the disease was fairly uniform in all. They showed enlarged and caseous regional lymph-nodes, spleen, and a few foci in the liver.

Result.—Probably little difference in the course of infection, but possibly the dose of tubercle bacilli was too large to indicate slight differences. Long cessation of treatment of the immunized cow may account for similarity to the control.

The results in the last experiment were confirmed by another eye inoculation nine months after the last treatment of Cow 5.

EXPERIMENT 237 H.—Nov. 11, 1907: *Corneal inoculations in rabbits.* Serums of Cows 5 and 16 bled Nov. 9, 1907. Four rabbits inoculated as before with twenty-four-hour sensitized bacilli H39 on opposite eyes.

Result.—After frequent observations for thirty-five days no differences were observed in the course of infection, which was gradual and mild throughout.

The injections of tubercle bacilli residue into Cow 5 were resumed Nov. 9, 1907, after a negative tuberculin test of 0.5 c.c. O. T. The doses were increased every three or four days from 5 c.c. to 35 c.c. December 20.

Another corneal inoculation was undertaken ten days later with the same technic previously described but using very few bacilli.

EXPERIMENT 237 I.—Dec. 31, 1907: *Corneal inoculations in rabbits,* with serums of Cows 5 and 16 bled December 30. Both agglutinated 1 to 50. Emulsion of H39 sensitized twenty-four hours and opposite eyes of ten rabbits were inoculated with a standard loopful of the respective sediments. Smears of one loop of each showed clumps of two to five bacilli in a field. Serum-cultures were also made but revealed no contaminations. Careful daily observations on the course of infection were recorded. The eyes of the rabbit inoculated with Serum 5 began to show inflammation first, twice as many having redness and conjunctival hyperemia in the region of the inoculation up to the tenth day. In the controls the redness was slight in seven eyes on the tenth day, while it was marked in the eyes of all but one of the rabbits inoculated with Serum 5. Seven of the latter also had distinct focal infiltration of leukocytes, but none of the controls showed it until after the tenth day.

After twenty-six days five of the Serum 5 eyes had a larger focus, pannus, or beginning ulcer. Only one of the controls was more diseased than its opposite when the experiment was terminated, the animals being killed by bleeding and puncture.

Result.—Clearly an acceleration of tuberculous infection by the inoculation of bacilli sensitized with specific serum.

The method here employed was more satisfactory in that a daily ocular inspection of the progressing disease was possible and the comparisons could be made on the same animal. The human bacillus having mild infectivity for the rabbit restricted the disease to the superior segment of the cornea and the animals betrayed discomfort only during the onset of the infection.

Period of Interrupted Immunization, Cow 5

Another year passed, during which time Cow 5 was under treatment with tubercle bacilli residue until June 11, 1908. Except for an experiment in January, 1908 (to be mentioned later), no further test of the serum was made until six months after the last treatment, when it was determined to repeat the corneal inoculation method to see if there was again a loss of specific effect.

EXPERIMENT 269.—Dec. 22, 1908: Six rabbits were inoculated with sensitized bacilli from Serums 5 and 16, using quantities of culture and technic as nearly identical with the previous test as was possible. The result after thirty days showed no perceptible difference between the eyes as to the course of disease. This confirmed our observation in 1907 that no specific effect was obtainable from Serum 5 so long after cessation of the injections of residue.

INCREASED PROTECTIVE INFLUENCE OF SENSITIZED TUBERCLE BACILLI AGAINST SUBSEQUENT INFECTION IN GUINEA-PIGS

In consideration of the enhanced infective power acquired by our weak virulent human culture R1 when sensitized by Serum 5 (Experiment 237 C2) it was of interest to observe what effect on subsequent virulent reinfection it would have. As in former experiments the R1 culture had shown a marked protection against reinfection with virulent inoculation, it was possible to expect it to be increased, or, on the other hand, it might lessen the resistance of the animals.

Cow 5 was continued under treatment from November, 1907, to June, 1908.

EXPERIMENT 263.—Jan. 8, 1908: Bled Cows 16 and 5 eight days after the last injection of 25 c.c. tubercle bacillus residue.

January 9: Serums centrifuged and added to emulsion of weak-virulent human culture R1 broth from Dec. 17, 1907, fresh growth. Took 0.030 gm. (moist) to 15 c.c. normal saline; centrifuged twenty-five minutes; 5 c.c. supernatant filtered through sterile paper and cotton several times and diluted to 10 c.c. Smears gave 5 or 6 tubercle bacilli per field.

A: 1.8 c.c. emulsion + 4 c.c. Serum 5 (active).
B: 1.8 c.c. emulsion + 4 c.c. Serum 16 (active).
C: 1.8 c.c. emulsion + 4 c.c. culture broth.

All incubated twenty-four hours; centrifuged A and B forty minutes; pipeted and replaced by 5.8 c.c. culture-broth to each; mixed all tubes thoroughly. Inoculated thirty guinea-pigs, ten for each preparation, with 0.1 c.c. in the left groin subcutaneously. Ten other pigs were selected for control to

the virulent inoculation. They were then kept for three months before reinoculation.

February 10, thirty-second day: Palpated and weighed. Series A, Pigs 3 and 7, died emaciated February 4; slightly enlarged nodes in left groin; no others have palpable enlarged nodes; all but one losing weight.

Series B, Pigs 1 and 7 died in January, chronic pneumonia. No signs of tuberculosis; no enlarged nodes; all but three gaining weight.

Series C, no deaths; no palpable enlarged nodes; all have lost weight.

Series D (controls): One died of chronic pneumonia February 17; all but three losing weight.

February 17 and 18: Pig 4A and 4C died of chronic pneumonia; no other disease evident.

March 5: Pig 10A died emaciated; no disease except left inguinal nodes larger than normal.

March 6: Pigs 1 and 4D died, the former only emaciated; the latter with large submaxillary abscess.

March 19: Pigs 5 and 6C, 6 and 11D were taken for another experiment.

March 20, sixtieth day: The twenty-six remaining animals were each inoculated in the right groin with 0.3 c.c. centrifuged, filtered emulsion human tubercle bacilli H37 broth culture March 3, 1908. Smears of emulsion show from 5 to 6 per field. Practically all the guinea-pigs had gained weight except Pig 6 of Series A and Pigs 3 and 6 of Series B.

April 5: Pig 8A died emaciated; no visible disease; both inguinal chains normal.

April 29: All killed for comparison except Pig 7A and Pig 10B (suckling young).

Of the four, Series A, none had any trace of disease in the left inguinal nodes, but showed some tuberculous involvement in the right; smears were tubercle-bacilli-positive from the last, but negative in the other, and also from the iliac nodes and spleens of all this series; otherwise normal.

Of the six of Series B, two had both inguinal nodes involved; all had right; two with abscesses in the left; two in the right; one with iliac, and the spleens of three were suspicious though in two smears were negative; otherwise normal.

Of the seven of Series C, there was a close resemblance to Series B in most of them, only in one the left inguinal node being enlarged.

Of the six controls, D, all had extensive, uniformly caseous nodes, spleen and liver, the latter in the first stages. The lungs were not diseased.

Pig 10B died emaciated a month later with no visible disease. Pig 7A lived until Feb. 19, 1909, when it succumbed to advanced fibroid tuberculosis, presumably from the virulent infection.

Result.—There was a slight difference in favor of the immune serum-sensitized animals as to the extent of the infection, it being less than any of the controls. None showed the amount of disease found in the normal-serum controls, though the latter had hardly reached beyond the inguinal-iliac chain of lymph-nodes.

There was an unusually large mortality at first in this experiment from chronic pneumonia, which detracted from its value in point of numbers. The result was, however, suggestive of a specific influence in aiding the protective inoculation.

Further Immunization of Cow 5

In consideration of the low agglutination titer and complement deviation that we had obtained with Serum 5, it was determined to try larger inoculations of human bacilli followed by tubercle bacillus residue before abandoning the search for bactericidal properties in the serum.

The dangers of intravenous inoculations containing clumps of bacilli from emboli in the brain and other organs necessitated caution.

In addition to this danger, which in one of our own calves and those of other experimenters had led to tuberculous abscesses in joints, there was danger of anaphylactic shock, which at that time was well recognized as such, though formerly misinterpreted. By sedimentation and dilution we hoped to avoid the first danger, and by moderate dosage, the second. In this we were successful, and during the next two years Cow 5 received four more inoculations of living, virulent human bacilli H37 besides intervening treatment with tubercle bacilli residue and other tuberculins.

The dates of inoculations were as follows:

Dec. 29, 1908: Third inoculation; 10 c.c. centrifuged emulsion, estimated (by deducting sediment) 0.060 gm.

August 5, 1910: Fourth inoculation; 25 c.c. centrifuged emulsion, estimated as previously, 0.170 gm.

Sept. 20, 1910: Fifth inoculation; 25 c.c. centrifuged emulsion, same estimation, 0.175 gm.

Dec. 30, 1910: Sixth inoculation; 25 c.c. centrifuged emulsion, estimated as before, 0.130 gm.

Fever reactions from 103 to 106 F. followed each dose within six hours, but subsided on the next day. The cow remained in fine flesh and condition throughout the further treatment, yet no reactions resulted from subcutaneous tuberculin injections of various kinds. Feb. 1, 1911, 1 c.c. O. T. diluted was given intravenously. There was respiratory embarrassment for ten minutes followed by a fever to 105.6 in six hours. Feb. 7, 1911, 1.5 c.c. O. T. was given in the same way. Marked difficulty and acceleration of respiration followed, with dizziness and a temperature of 104.5 in six hours.

The symptoms of anaphylaxis were too well marked to permit further intravenous injections. Further subcutaneous injections of W. E. and T. O. were given, but produced no higher agglutination titer than 1 to 100. A slight precipitation reaction was obtainable. The complement deviation was also found no lower in many tests with the same stock antigen. The titer during 1910 and 1911 was 0.02 c.c. serum to completely absorb complement with 0.1 c.c. T. R. emulsion (0.001 gm. dry to 1 c.c.). Considering the unpromising outlook for a higher grade of antibodies the immunization was discontinued April 27, 1911.[13] The animal was still in perfect condition when slaughtered in December, 1912. Very unfortunately no post-mortem examination was feasible, as she was kept at a distance from the town.

Final Experiments to Test Bactericidal Effect of Serum, Cow 5

EXPERIMENT 206 A.—June 23, 1909: *Subcutaneous Inoculations.* Six months after the third reinoculation, during which time continuous treatment with tubercle bacilli residue was carried on. Bled Cows 5 and 16.

July 27: Incubated with emulsion virulent human culture H41 (prepared as in previous experiments) four days without change. Centrifuged; inoculated subcutaneously into five pigs for each test in right inguinal region. Serum 5 animals died sooner than those of Serum 16, the average being 36.2 days as compared with 55.2 days for the controls.

The Serum 5 animals emaciated and revealed disease in the inguinal and iliac nodes with caseation or beginning abscess. One survived three months

13. It must be confessed that we dropped this work at this point because other things seemed more important, and it was impossible to keep large animals near the laboratory with our limited facilities.

and had an abscess in the groin with spleen and lungs involved. The controls that survived longer had more disease and three showed caseous nodes and foci in the spleen.

Result.—No specific effect of Serum 5 was demonstrated in this experiment unless the shorter average lifetime of the Serum 5 animals may be ascribed to some toxic action produced by the serum acting on the tubercle bacilli and which was liberated in the animals. The animals were in poor condition at any rate.

A final attempt to show a bactericidal influence of Serum 5 was made a month after the sixth and last inoculation, and a week after a tuberculin reaction on Feb. 1, 1911.

EXPERIMENT 206 B.—Feb. 7, 1911: *Intracutaneous inoculations.* A different method was tried in this experiment by comparing intracutaneous inoculations made on the same animal simultaneously. A new normal cow was used for control serum, and both active and inactive serums used, together with a broth control. Opposite sides of six guinea-pigs were shaven and the inoculations made after three days' incubation with human tubercle bacilli H37. The inoculations were made sufficiently far apart to cause any marked difference in the reactions to be noticeable. The infections were practically the same up to the nineteenth day, when all but the broth-controls began to show caseous centers. The Serum 5 (active) seemed more inclined to ulcerate than the rest, yet the infiltration and redness were practically alike. At this time no lymph-nodes showed enlargement. As no certain differences were assured the animals were soon killed.

Result.—No evidence of specific action from Serum 5 in this experiment.

DISCUSSION

The general effect noted throughout the foregoing experiments was unfavorable so far as any practical value of the immunized cow-serum in the therapy of tuberculosis.

The sensitized living bacilli which we hoped might be weakened by the serum were in fact rather more infective and therefore less available for vaccines.

The phenomenon of increased infectivity was associated with serums from the cow under active immunization with T. R. During resting periods the serum lost its property for the time, but soon regained it when the injections of T. R. were resumed. The antibody reactions were never strong, and the agglutination was the most delicate and pronounced of any. The serum titer was, notwithstanding, often no higher than that from a normal cow, so that it was difficult to understand that the difference in infectivity was due to the greater agglutination. Considering the claim that serum-sensitized B. E. tuberculin is more easily absorbed than the plain emulsion, one may account for the increased virulence produced in guinea-pigs and rabbits by the bacteriotropic effect of the serum on living bacilli as well as by the agglutination.

It may be that specific agglutinins and bacteriotropins excite a more active phagocytosis under these conditions. The leukocytes ingest large clumps of agglutinated bacilli which they are unable to dispose of. In the non-sensitized bacilli they find them in singles and can weaken

or kill them more readily. As a working theory the preceding explanation, partly mechanical as well as biological, may account for our results. In many other pathogenic organisms one may note rapid lysis under the influence of antiserums or in similarly sensitized bacteria when inoculated. Even were an active lysin present in our cow-serums the difficult solubility of the tubercle bacilli undoubtedly masks it.

It has in fact been suggested that by prolonged digestion of living tubercle bacilli in active immune serums (as in our experiments) the weaker bacilli are devitalized, if not dissolved, leaving only the most vigorous and resistant ones. The latter when inoculated, being clumped together, are able to survive the cellular digestion longer. In these ways one may explain the seeming paradox in our results and the causes for the earlier reaction and further spread of the disease in the inoculated animals.

Intracellular digestion of tubercle bacilli is slow when observed in the peritoneal exudate of previously infected animals. It is much slower in control animals and few observers have claimed to dissolve them at all in immune serum *in vitro*. The peculiarity of the wax in tubercle bacilli places this organism in a class by itself as unusually resistant against lysis. Under the most favorable conditions for the lysis of tubercle bacilli a few often escape and may produce an infection; hence the difficulty of experimental demonstration of the mechanism.

Some consideration should be given to the possible influence of the cow-serum as such in our experiments. In most cases it was washed out of the prepared bacilli and only the antibody fixed to the bacilli could come into the question. There is no reason to think that it caused any aggravation of the infection, except through the anchored antibody. It is true that other observers (Calmette and Guérin[14]) have found that immunized cow-serum aggravated the disease in guinea-pigs, though in a recent tentative experiment Rothe and Bierbaum[15] on two guinea-pigs thought that 0.1 to 0.2 c.c. immunized cow-serum mixed with tubercle bacilli twenty hours weakened the infection. Immunized cow-, horse- and mule-serum were found by Ruppel and Rickman[16] to restrain infection in guinea-pigs by injections of 5 c.c., though it is not stated which serum was used, nor have further details been published.

Nearly all of the earlier experiments were with repeated injections of horse-serum or that of other immunized animals: Bâbes and Proca,[17]

14. Calmette and Guérin: Ann. d. l'Inst. Pasteur, 1911, xxv, 626.
15. Rothe, E., and Bierbaum, K.: Veröffentl. d. Robert Koch Stiftung 1913, Nos. 8, 9, p. 169.
16. Ruppel and Rickman: Ztschr. f. Immunitätsforsch., 1910, vi, 383.
17. Bâbes, V., and Proca, G.: Ztschr. f. Hyg. u. Infectionskrankh., 1896, xxiii, No. 3, p. 331.

Karwacki.[18] They were usually harmful because of the anaphylactic sensitization produced, which at the time was not understood.

In all these experiments with antituberculous serums it must also be remembered that an anaphylactic antibody to tuberculo-protein must frequently be present in the serum. This might be a source of slow poisoning to a tuberculous animal by acting on the bacilli and setting free small amounts of anaphylatoxin. It is conceivable that the early reaction and greater irritation of the rabbit cornea seen in our experiments with immune-serum-sensitized bacilli were due to this cause. The probability of this is lessened by the fact that the poison would have been equally strong, if not stronger, from the bacilli sensitized with inactivated than with the active serum.

To recapitulate the conclusions of most importance, the following summary may be presented:

SUMMARY

1. The foregoing experiments relate to the properties of the serum of a cow repeatedly immunized against tuberculosis during a period of ten years.

2. Six intravenous inoculations of living human cultures of tubercle bacilli, both of virulent and weak-virulent infectivity, besides numerous injections of all forms of tuberculin, were given to this cow.

3. Specific agglutinin, precipitin, opsonin, and complement-deviating antibody were demonstrated in varying degree in the serum but never reached a high content.

4. The presence of a bacteriolysin was not demonstrated; neither was a bacillicidal effect recognized either in the serum or leukocytes.

5. Living human tubercle bacilli "sensitized" with the immune serum showed increased infective power in guinea-pigs and rabbits as compared with normal cow-serum.

6. The increased infective power was manifested by earlier and more marked reactions or inflammation following inoculation, and a wider spread of the disease than in controls.

7. This acceleration of infection was not manifest unless the cow had recently received injections of pulverized bacillus residue, but the immune-serum-agglutinin titer was not always greater than the normal cow serum.

8. To explain the apparent paradoxical action of the immune serum, it is suggested that the strongly agglutinated bacilli were protected from injury by the leukocytes of the inoculated animal by reason of the clumping. The bacilli were presumably phagocyted in large masses but more difficult of digestion for that reason. It may also be suggested that the

18. Karwacki, L.: Ztschr. f. Tuberk., 1906, viii, 52-59.

bacilli laden with antibody (opsonin) were more rapidly phagocyted and scattered, but owing to the resistant wax were not digested in sufficient numbers. ,

9. A "sensitized" *living* tubercle-bacillus vaccine is not safe or practicable when prepared from immune bovine serums, considering the above-mentioned results.

My heartiest acknowledgements are due, especially to Dr. Joseph L. Nichols, Dr. J. Woods Price, and the late Dr. Albert H. Allen, who assisted in these studies.

The expense of this work was borne by Mrs. A. A. Anderson of New York City, to whom we also owe our thanks.

AN EXPERIMENTAL STUDY OF INTESTINAL OBSTRUCTION *

JOHN A. HARTWELL, M.D., J. P. HOGUET, M.D.
AND
FENWICK BEEKMAN, M.D.
NEW YORK

Intestinal obstruction in man, if unrelieved, speedily causes death. The fatal outcome is too rapid to be the result of starvation, and three general theories have been advanced to explain it:

1. A disorder of the nervous mechanism controlling the cardiac and vasomotor systems.

2. A bacterial infection of the organism by the passage outward of bacteria from the intestinal lumen.

3. An intoxication from poisonous substances imprisoned in the intestine orally to the obstruction.

Two modifications of the last theory have recently come into prominence. The first was originally advanced by Vidal,[1] and more recently elaborated by Maury Draper[2] (formerly Draper Maury), in which the presence of a substance in the upper intestinal tract was believed to exist, which, unless neutralized by substances elaborated lower down, entered the system as a poison.

The second modification is advanced by Whipple, Stone and Bernheim.[3] These workers believe that stagnation of the duodenal contents results in the formation of a severe poison, which readily enters the system, and produces death. This poison may be obtained from the mucosal cells of the duodenum as well as from the duodenal contents.

THE THEORY OF A NERVOUS DISORDER

In favor of this theory, clinical evidence has been adduced. The tachycardia, the low blood-pressure, the profound collapse of the patient,

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* Preliminary reports on the work here detailed have appeared in Am. Jour. Med. Sc., March, 1912, p. 357; Jour. Am. Med. Assn., 1912, lix, 82; Jour. Exper. Med., 1913, xviii, No. 2, p. 139. These reports are freely abstracted in the present article in order to present the subject in its entirety.

1. Vidal: Rev. de chir., 1900, xxvi, 521; Cong. Franc. de chir., 1905, xxviii, 1237.

2. Draper, J. W.: Experimental Intestinal Obstruction, Jour. Am. Med. Assn., 1911, lvii, 1338; John Hopkins Hosp. Bull., 1909, xx, 184; Am. Jour. Med. Sc., 1909, cxxxvii, 725; Death in Acute Intestinal Obstruction and Kindred Conditions Is Due to Physiologic Disturbance, Jour. Am. Med. Assn., 1910, liv, 5.

3. Whipple, Stone and Bernheim: Jour. Exper. Med., 1913, xvii, No. 3, p. 286.

and the wide dilatation of the splanchnic blood-vessels, all point to a loss of activity on the part of the medullary nerve-centers. The early writers saw in these the results of a reflex disturbance from stimuli acting on the nerve endings in the intestinal wall. It is undoubtedly true, however, that exactly this train of events may arise from a toxemia or a bacteriemia without the intervention of any afferent nerve impulses.

Recently, Braun and Boruttau[4] have modified this theory somewhat, and assign the above-described symptoms to a disturbance of the circulation resulting from interference, chiefly mechanical, with the inherent nerve plexuses in the intestinal wall. They see a parallel between a gradual bleeding to death and the death in intestinal obstruction. The loss of body fluids resulting from failure of absorption, the enormous outflow of fluid into the intestine, the persistent vomiting, and the stagnation of blood in the splanchnic area lead to an anemia of the brain-centers which progresses till these centers cease their activity, and death results. They experimented most exhaustively on rabbits to support this view, but the evidence is all indirect, and seems to us not to amount to a proof of the theory. We are unable to find in the literature any convincing experimental work that the nervous system is primarily at fault. Certainly, as death approaches, there is a profound disturbance of the nervous control of the heart, blood-vessels and respiration, but this, we believe, is in no sense a reflex disturbance, nor the result of an anemia, *per se*, of the nerve-centers. It differs in no way from the last stages of any severe sickness which is progressing toward a fatal issue. The loss of body fluids is a matter of the utmost importance, but not because of its effect in producing a brain anemia.

THE THEORY OF BACTERIAL INFECTION

The infection theory has much more to support it. The intestinal lumen is the normal habitat of innumerable micro-organisms which are potentially pathogenic. When an obstruction exists, these no longer have their normal outlet per anum. They are dammed up in the intestine under favorable conditions for their growth (McClure[5]). The damage to the intestinal mucosa may allow their passage outward, either into the blood-stream, lymphatics, or directly into the peritoneal cavity. Proof of this possibility is seen clinically in the presence of a peritonitis as a complication of intestinal obstruction without perforation; also in the frequent finding of colon bacillus in the blood as a pathogenic micro-organism, under very varied conditions of intestinal disturbances less severe than an obstruction, and in the bacteriemia from the *Bacillus typhosus* when the intestine is ulcerated by the action of that organism.

4. Braun and Boruttau: Deutsch. Ztschr. f. Chir., 1908, xcvi, 544.

5. McClure, R. D.: An Experimental Study of Internal Obstruction, Jour. Am. Med. Assn., 1907, xlix, 1003.

Von Khantz,[6] experimenting on rabbits in a low intestinal obstruction, often found a bacterial invasion of the blood and the peritoneum, but never the former without the latter. Borszesky and von Genersich,[7] on the other hand, found a blood infection with no invasion of the peritoneum. There can be no doubt that a bacterial invasion of both the peritoneum and the blood may take place in intestinal obstruction, but it is not an essential accompaniment. It occurs late as a complication, often a terminal one. Both clinically and experimentally it will result most often when the obstruction is complicated by a strangulation. Without this complication, intestinal obstruction will kill with no passage of bacteria beyond the intestinal wall.

Tables 1 and 2 give the results of our experiments bearing on this point. In the first series the obstruction was produced by dividing the intestine from 10 to 30 cm. below the pylorus, and closing the ends by inversion. It will be noted that in all these cases some micro-organisms were found in the organs examined. This, we believe, was due to the fact that all the examinations were made several hours post mortem, because all the animals died during the night, and possibly to a local soiling at the site of operation. In the second series, however, the obstruction was produced by the clamp described below, and the animals were either killed when it was believed they were too sick to live over night, and immediately examined, or examined very soon after death occurred from the obstruction itself. This series of seven cases furnishes irrefutable proof that death results from an obstruction of the upper intestine in the dog, without any invasion of the peritoneum, blood, liver or spleen by bacteria, which are demonstrable by the methods employed. The methods in the two series were exactly similar and their efficiency in detecting organisms is amply demonstrated in the first. The cultures were taken at autopsy on slant agar or Loeffler blood-serum, or both. Usually four tubes, sometimes only three of each, were inoculated from each tissue, and grown under both aerobic and anaerobic conditions at incubator temperature. For the anaerobes the Buchner method or the Cornell modification of it was used. The tubes were examined at the end of one, two and three days. All the cultures were made in the sterile room of the Cornell bacteriology laboratory. This consists of a closed cabinet, ventilated through a bacteria-proof filter, in the roof of which is suspended an ordinary revolving lawn-sprinkler. The water is turned through this for fifteen or twenty minutes prior to taking the cultures. Thus the air is mechanically sterilized by washing out the micro-organisms. All the needed apparatus for taking the cultures and smears is assembled before beginning the inoculations,

6. Von Khantz: Arch. f. klin. Chir., 1909, xxxviii, 412.
7. Borszesky and von Genersich: Beitr. z. klin. Chir., 1902, xxxvi, 448.

and the air-tight door is kept closed until all cultures are completed. The technic in making the inoculations is that usually employed in obtaining cultures from post-mortem material. The efficiency of the room and the method is demonstrated by the fact that only three air contaminations were found in several hundred tubes.

In some of the later cases of the second series no cultures were taken from the spleen, since it was noted that this organ never showed infection when the liver and peritoneum were without infection. Often, too, the spleen cultures gave no growth when the other organs were actively infected. A summary of this series shows that the femoral blood was cultured seven times, and always was without growth; the liver was cultured six times, and showed one colony of Gram-positive cocci, in one tube of one case, probably an air contamination; the results from the peritoneal cultures were exactly the same as from the liver; the spleen was cultured only twice, with negative findings both times; the jejunal or duodenal contents above the clamp were cultured seven times, and all tubes showed an abundant growth of various micro-organisms.

TABLE 1.—CULTURES FROM ORGANS OF ANIMALS WITH INTESTINAL OBSTRUCTIONS, SERIES 1

Case	Organs	Cultures Taken	Tubes Showing Cocci	Tubes Showing Bacilli	Characteristics	Remarks
12	Spleen	2 aerobic 3 anaerobic	None	1 aerobic 2 anaerobic	Morphology and growth characteristic of colon bacillus on usual differential mediums.	Intestine sectioned and closed 34 cm., distal to pylorus. Lived 80 hours. Cultures taken 10 hours post mortem
	Liver	2 aerobic 4 anaerobic	All	All	(1) Gram + coccus. (2) Bacillus identified as colon on differential mediums. (3) Also very many colonies of a large Gram + anaerobic bacillus with morphology of *Bacillus aërogenes capsulatus.*	
	Jejunum	2 aerobic 4 anaerobic	All	All	(1) Gram + coccus. (2) Large anaerobic Gram + bacillus. (3) Gram — bacillus of two kinds, first long and slender; second, short and plump. Both grew in anaerobic tubes, while the former only grew in aerobic tubes.	
13	Femoral blood	4 each	None	None	.....	Intestine sectioned 36 cm. from pylorus and ends closed. Autopsy about 13 hours post mortem.
	Peritoneum	4 each	All	None	(1) Gram + and (2) Gram — coccus.	
	Spleen	4 aerobic 3 anaerobic	1 aerobic	None	Gram + coccus. Same organism as in peritoneum.	
	Liver Jejunum	4 each 4 each	None All	None All	Same as in Case 12.	Cultures taken from jejunum when it was sectioned at operation gave the same results as at autopsy.

TABLE 1.—CONTINUED

Case	Organs	Cultures Taken	Tubes Showing Cocci	Tubes Showing Bacilli	Characteristics	Remarks.
14	Jejunum at operation	2 aerobic 2 anaerobic	All	All	(1) Gram — bacillus. (2) Gram + coccus. (3) Large Gram + bacillus.	Intestine sutured after section 31 cm. from pylorus. Autopsy 36 hours post mortem.
	Jejunum at autopsy	4 each	All	All	(1) Anaerobic gas-forming Gram — bacillus. (2) Gram + bacillus. (3) Gram + coccus.	
	Femoral blood	4 each	2 aerobic	All	(1) Gram + coccus, ferments lactose, and glucose, without gas, no change in neutral red and saffranin. (2) Large anaerobic Gram + bacillus with morphology of aerogenes capsulatus bacillus.	
	Peritoneum	2 each	All	All	(1) Gram + coccus, same as in femoral blood. (2) Gram — bacillus-like colon bacillus.	
	Spleen	4 each	2 aerobic	2 anaerobic	(1) Gram — coccus. (2) Gram — bacillus having the characteristics of colon bacillus.	
	Liver	2 anaerobic 4 aerobic	2 anaerobic 3 aerobic	None	(1) Gram + coccus as in peritoneum and femoral blood.	
15	Jejunum at operation	4 each	4 anaerobic	4 anaerobic 2 aerobic	Gram — bacillus, mobile same as in peritoneum.	Intestine ligated with tape 35 cm. from pylorus. Autopsy 4 hrs. post mortem. General peritonitis present.
	Jejunum at autopsy	4 each	1 anaerobic	All		
	Peritoneum	4 each	4 anaerobic	All	(1) Gram — bacillus having characteristics of colon bacillus (2) Gram — bacillus intermediate in colon group, changes neutral red and saffranin, does not ferment lactose. (3) Gram + coccus.	
	Femoral blood	4 each	None	None		
	Spleen	4 each	None	None		
	Liver	4 each	None	None		
		4 each				
16	Jejunum at operation		.....	All	Not identified.	Bowel sectioned and inverted 48 cm. from pylorus. Lived about 32 hours. Autopsy 12 hours post mortem. No peritonitis.
	Jejunum at autopsy	4 each	All	All	(1) Short Gram — bacillus. (2) Gram + coccus resembling streptococcus. (3) Large Gram + bacillus.	
	Femoral blood	4 each	None	None		
	Spleen	4 each	None	None		
	Liver	4 each	All	All	Gram — short, stout, bacillus mobile (2 strains), resembling colon bacillus. (2) Gram + bacillus. (3) Gram + coccus.	
	Peritoneum	4 each	All	All	(1) Gram + coccus. (2) Gram + bacillus. (3) Gram — bacillus, short and stout, mobile. Same as in liver.	

TABLE 1.—CONTINUED

Case	Organs	Cultures Taken	Tubes Showing Cocci	Tubes Showing Bacilli	Characteristics	Remarks
17	Jejunum at autopsy	None taken	None taken	.....	.....	Jejunum clamped 37 cm. from pylorus with Crile clamp, which at autopsy was found to have cut through. Autopsy 8 hours post mortem, lived about 88 hrs. General peritonitis present.
	Femoral blood	4 each	None	None		
	Spleen	4 each	1 anaerobic	None	Gram + coccus. One colony outside line of inoculation. Probable air contamination.	
	Liver	4 each	None	None		
	Peritoneum	4 each	4 anaerobic 3 aerobic	4 anaerobic 3 aerobic	(1) Gram — bacillus, having characteristics of coli communis. (2) Gram + coccus. (3) Gram + bacillus short and fat and growing in long chains.	
18	Jejunum	None taken	.....	.....	.....	Jejunum clamped with Crile clamp 20 cm. from pylorus. Killed with chloroform 91 hrs. after operation. Clamp cut through, local peritonitis resulting.
	Femoral blood	4 each	None	None	(1) Gram — bacillus.	
	Peritoneum	4 each	None	All	(2) Gram + bacillus.	

TABLE 2.—CULTURES FROM ORGANS OF ANIMALS WITH INTESTINAL OBSTRUCTIONS, SERIES 2

Case	Organs	Cultures Taken	Tubes Showing Cocci	Tubes Showing Bacilli	Characteristics	Remarks
19	Jejunum	3 aerobic 3 anaerobic	3 anaerobic	3 anaerobic 3 aerobic	(1) Gram — bacillus. (2) Gram + bacillus.	Intestine occluded with special clamp 18 cm. from pylorus. Lived 172 hrs. after operation. Killed with chloroform. Autopsy immediately. No peritonitis; local abscess around clamp.
	Femoral blood	4 aerobic 3 anaerobic	None	None		
	Spleen	4 aerobic 4 anaerobic	None	None		
	Liver	4 aerobic 3 anaerobic	None	None		
	Peritoneum	3 anaerobic 4 aerobic	None	None		
	Abscess around clamp	2 aerobic 2 anaerobic	.....	1 anaerobic 2 aerobic	Gram — bacillus.	
20	Jejunum	None taken	.....	.....	.....	Occlusion clamp 16 cm. from pylorus. Lived 90 hrs. Died. Autopsy. No peritonitis.
	Femoral blood	4 aerobic 4 anaerobic	None	None		
	Liver	4 anaerobic 4 aerobic	None	None		
	Peritoneum	4 aerobic 4 anaerobic	None	None		
	Exudate around clamp	3 aerobic 3 anaerobic	None	None		
21	Jejunum	4 aerobic	4 anaerobic	4 anaerobic	(1) Gram + coccus. (2) Gram + bacillus. (3) Gram — bacillus.	Occlusion clamp on jejunum 16 cm. from pylorus. Dog lived 58 hrs. Autopsy 8 hrs. post mortem. Peritoneum clean.
		4 anaerobic	4 aerobic	4 aerobic		
	Femoral blood	4 aerobic 4 anaerobic	None	None		
	Spleen	4 aerobic 4 anaerobic	None	None		
	Liver	4 aerobic 4 anaerobic	None	None		
	Peritoneum	3 aerobic 4 anaerobic	1 aerobic	.....	Gram + coccus. One colony only, probably air contamination.	

TABLE 2.—CONTINUED

Case	Organs	Cultures Taken	Tubes Showing Cocci	Tubes Showing Bacilli	Characteristics	Remarks
22	Jejunum	3 aerobic 3 anaerobic	2 aerobic 1 anaerobic	.....	Gram + cocci.	Occlusion clamp 12 cm. from pylorus. Lived 21 days. Killed with ether. Clamp not absolutely tight. No peritonitis.
	Femoral blood	4 aerobic 4 anaerobic	None	None		
	Liver	3 aerobic 3 anaerobic	None	None		
	Peritoneum	4 aerobic 4 anaerobic	None	None		
23	Jejunum Liver	None taken None taken	.....	.....	.....	Occlusion clamp 12 cm. from pylorus. Lived 240 hrs. Killed with cyanid and autopsied immediately. No peritonitis. Clamp tight.
	Femoral blood	3 aerobic 4 anaerobic	None	None		
	Peritoneum	4 aerobic 4 anaerobic	None	None		
24	Jejunum	2 aerobic 2 anaerobic	None	2 aerobic 2 anaerobic	Gram + and — bacilli	Occlusion clamp 16 cm. from pylorus. Lived 205 hrs. Autopsied 2 hrs. post mortem. No peritonitis. Small abscess around clamp was tight.
	Femoral blood	3 aerobic 4 anaerobic	None	None		
	Liver	4 aerobic 4 anaerobic	None	None		
	Peritoneum	4 aerobic 4 anaerobic	None	None		
	Clamp abscess	2 aerobic 2 anaerobic	2 anaerobic 2 aerobic	None	Pure growth of Gram + coccus.	Occlusion clamp 16 cm. from pylorus. Lived 240 hrs. Killed with ether. Autopsy immediate. No peritonitis.
26	Jejunum	4 aerobic 4 anaerobic	..... None	4 aerobic 4 anaerobic	(1) Small Gram — bacillus. (2) Large, long Gram + bacillus.	
	Femoral blood	4 aerobic 4 anaerobic	None	None		
	Liver	3 aerobic 4 anaerobic	1 aerobic	None	One colony outside inoculation of a Gram + coccus.	
	Peritoneum	4 aerobic 4 anaerobic	None	None		
28	Jejunum	2 aerobic 2 anaerobic	2 anaerobic	2 aerobic 2 anaerobic	Gram + and — bacilli and cocci. Anaerobic tubes show gas production.	Occlusion clamp 11 cm. from pylorus. Lived 56 hrs. Autopsy 10 hrs. post mortem. Abscess around clamp. No peritonitis. Clamp tight.
	Femoral blood	4 aerobic 4 anaerobic	None	None		
	Liver	4 aerobic 3 anaerobic	None	None		
	Peritoneum	4 aerobic 4 anaerobic	None	None		
	Clamp abscess	2 aerobic 2 anaerobic	None	None		

Case 22, given in Table 2, is not included in the summary because the obstruction was not complete. It is worthy of note here, however, that the jejunal tube only showed a growth of a Gram-positive coccus in one-half the tubes inoculated, showing that the long period (twenty-one days) with only sterile water by mouth did much toward sterilizing the upper bowel.

It is thus demonstrated that no matter what the rôle of the micro-organisms may be within the bowel, or no matter how active they may sometimes become in causing complications by penetration of the bowel into the general system, this is not a necessary factor either in causing

the symptoms of the disease or in determining the fatal outcome. The theory, therefore, which holds a bacteriemia as the responsible factor in the death following intestinal obstruction is untenable.

THE INTOXICATION THEORY

This theory, on the contrary, has experimental evidence to support it, and no experiments have proved the absence of a toxemia. Clairmont and Ranzi[8] demonstrated that the stagnated material above an obstruction, after passage through a Reichel or Pukal filter, was exceedingly toxic when injected into the blood of an animal. Kukula[9] found a toxic material in the alcoholic extracts of the intestinal contents in experimental ileus. Roger,[10] and Roger and Garnier,[11] went farther and proved that the normal content of both the stomach and the intestine are poisonous when thus injected. They did not filter it, but death occurred too early to be the result of an infection. The toxicity was only one-third as great when injected into the portal vein as when injected into a peripheral vein. They also injected it into a mesenteric artery, against the current, so that it was carried backward and then redistributed through branches to a considerable section of the intestine. Here it produced emboli and stopped the absorption, so that large doses were not fatal.

Murphy and Vincent[12] found that the material from the obstructed or strangulated intestine was very poisonous when injected into the peritoneal cavity, and that its poisonous properties were destroyed by boiling or passage through a Berkefeld filter. They concluded, therefore, that living bacteria are the important factors. It does not seem to us that this conclusion is tenable. Their experiments only prove that living bacteria, if they obtain entrance into the peritoneum, will kill.

Whipple, Stone and Bernheim[3] worked with a material obtained from the doubly occluded duodenum, and determined that it was very poisonous even after being rendered sterile. They also found what they believed to be the same poison in the mucosa of this part of the intestine, when one end of it was closed and the other end allowed to drain externally. They applied their results to a simple intestinal obstruction, and concluded that death in this condition is caused by a poison arising from the duodenal mucosa. Their views are opposed in important particulars to the results obtained by us, and will receive closer study later. In general it may be stated that the symptoms aris-

8. Clairmont and Ranzi: Arch. f. klin. Chir., 1904, lxxiii, 696.
9. Kukula: Arch. f. klin. Chir., 1901, lxiii, 773.
10. Roger: Rev. Scient., 1907, xlv, 65; Presse méd., 1911, xix, 1.
11. Roger and Garnier: Arch. de méd. expér., July, 1906; Rev. de méd., 1906, xxvi, 609; Presse méd., 1906, xiv, 325.
12. Murphy and Vincent: Boston Med. and Surg. Jour., 1911, clxv, 684.

ing from the injection of intestinal contents into the blood, as reported, differ very markedly from those appearing in the obstructed animal, and there are many objections, as pointed out by Guibe,[13] Braun and Boruttau,[4] Korentchevsky,[14] and others, against accepting these experiments as directly bearing on the cause of death in intestinal obstruction. The lessened absorption from the obstructed intestine is one objection offered. Clairmont and Ranzi[8] found that potassium iodid placed in the bowel above the obstruction was excreted from the kidneys in greater quantities than normal, up to eight or ten hours after the obstruction was produced; the excretion rapidly decreased after that, and came to a stop in about fifteen hours.

Braun and Boruttau[4] gave strychnin to their obstructed animals, and from the resulting symptoms they concluded that absorption became markedly reduced from the start. On this experiment they base their chief objection to the intoxication theory. A more important objection to the use of the evidence of the injection experiments as a proof of the intoxication theory is that many substances injected directly into the circulation are poisonous, whereas, if they have to pass through living cells to get into the blood, their poisonous properties are entirely destroyed. Peptone is such a substance, and is a good illustration in this connection. The injection of peptone into the blood produces symptoms of poisoning, which may end in death. The absorption of peptone from the intestine is, on the contrary, a normal physiologic process. Therefore, the mere presence in the obstructed intestinal tract of substances which act as poisons when injected into the blood is no indication that these substances cause the death resulting from an obstruction, since such substances may be normally found in the intestine in conditions of continued health. To accept the view that such a death results from a toxemia it must be conceived either that new poisons are elaborated and absorbed or that an abnormal absorption of the poisons normally takes place.

In undertaking our work, after a study of the literature, we tentatively accepted the first hypothesis and planned our experiments to determine by a process of exclusion from what sources conception was forced on us, namely, *that the essential factor in causing the symptoms and death in intestinal obstruction does not lie in the poisons per se, but in the production of lesions which favor their abnormal absorption, and lacking these lesions the animals die only from dehydration, or starvation if dehydration is prevented.* We believe our reader will get a clearer understanding of our findings if he will follow, step

13. Guibe: Presse méd., 1909, xvii, 233.

14. Korentchevsky: Roussky Vratsch, 1908, p. 1572. (Cited by Guibe, Note 13).

by step, the reasoning and experiments which led to this change of conception in our minds.

In searching for the source of the poison we accepted three possibilities: (1) foodstuffs or substances derived from them; (2) secretory substances from the alimentary tract and digestive glands, or their derivatives; (3) true bacterial toxins. It was our intention to exclude one after the other of these possible sources and study the conditions resulting after an obstruction was produced. It is readily seen that there may enter into all of these the result of bacterial activity. While, as detailed above, our bacterial studies showed that a bacteriemia is not necesasrily present, they did not exclude either a bacterial toxin or substances developed by virtue of the presence of bacteria in the occluded bowel.

EXPERIMENTS TO DETERMINE THE EFFECT OF FOODSTUFFS OR THEIR DERIVATIVES ABOVE THE POINT OF OBSTRUCTION

All animals were fully etherized before the operation. No food was given in any case after the operation, unless so stated. Water, however, was given freely, unless otherwise noted.

EXPERIMENT 3.—A cat which had received no food for ninety-six hours before operation. Duodenum sectioned and ends closed by inversion 10 cm. below pylorus. For three days the animal remained in good condition. Vomited. Fourth day showed weakness and some rigidity of muscles of extremities. Just before death had convulsions involving extremities. Killed with chloroform ninety-six hours after obstruction. Autopsy immediate. No peritonitis. Closure of bowel ends tight. Stomach contained small amount of normal-appearing gastric content. Duodenum empty. Neither stomach or duodenum was distended.

EXPERIMENT 4.—Fox terrier. Fed twenty hours before operation. Repetition of Experiment 3, the section being 11 cm. distal to the pylorus. Except for some weakness and vomiting, seemed like normal dog for four days. Died with progressive weakness and vomiting on the seventh day. Autopsy. No intestinal leakage. No peritonitis. Stomach slightly distended. Duodenum not distended.

EXPERIMENT 5.—Bitch. Weight 9.8 kg. Fed last time forty-eight hours before operation. Repetition of Experiment 3. Postoperative course the same. Lived one hundred hours. Autopsy. No intestinal leakage. No peritonitis. No distention of stomach or duodenum.

EXPERIMENT 6.—Bitch. Weight 10.85 kg. Food withheld for seventy-two hours. Repetition of Experiment 3, section being 12 cm. below pylorus. Postoperative course the same. Temperature varied from 36.6 to 39 C. Lived for fifty-five hours. Autopsy. Same findings as those given above.

EXPERIMENT 7.—Dog. Weight 19.7 kg. Fed last time forty-eight hours before operation. Same operation 16 cm. below pylorus. Some peritoneal soiling with intestinal contents. Postoperative course same as others, except more acute. Temperature as high as 39.9 C. Lived only thirty-six hours. Autopsy yielded same findings as others.

EXPERIMENT 8.—Dog. Medium weight. Fed six hours before operation. Same operation 12 cm. below pylorus. Postoperative course the same. Temperature 39 C. Lived one hundred and eighteen hours. Autopsy. Same findings and in addition there were noted three superficial areas in the duodenum which resembled ulcers.

EXPERIMENT 9.—Leakage of intestinal contents at closed end of proximal gut.

EXPERIMENT 10.—Same.

EXPERIMENT 11.—Dog. Weight 5.3 kg. Fed twenty-six hours before operation. Same operation 33 cm. below pylorus. Lived only ten to fourteen hours. Autopsy. No leakage. No peritonitis. Stomach was not distended. Mucosa showed small punctuate hemorrhages. Intestine proximal to section and for 80 cm. distal to it was much congested and showed hemorrhagic areas in it. We are unable to explain these last post-mortem findings as being the result of the operation. Our belief, however, is that the early death resulted because of them.

EXPERIMENT 12.—Bitch. Weight 9.2 kg. Fed twenty-six hours before operation. Same operation 36 cm. below pylorus. Postoperative course as above. Lived eighty-two hours. Autopsy. Same findings as in other cases.

EXPERIMENT 13.—Dog. Weight 8.2 kg. Fed twenty-six hours before operation. Bowel sectioned 36 cm. below pylorus and ends inverted. Symptoms same as others. Temperature 35.5 C. Pulse 180 after twenty-four hours. Lived thirty-eight hours. Autopsy. No peritonitis. Ends of bowel tight. At distal closure an intussusception 14 cm. long had taken place and the intestinal wall was much damaged. This, we believe, contributed to an early death.

EXPERIMENT 14.—Young dog. Weight 5.75 kg. Fed seven hours before operation. Bowel sectioned and ends inverted 31 cm. below pylorus. Never fully recovered from operation and died in twelve hours. Autopsy. No peritonitis. Closed ends tight.

EXPERIMENT 15.—Dog. Weight 10.5 kg. Fed forty-eight hours before operation. Bowel sectioned and ends closed 48 cm. below pylorus. Postoperative symptoms same as in other dogs. Lived thirty-two hours. Autopsy. No peritonitis. Closed ends tight; 10 cm. intussusception of distal end in gut, with much damage of gut wall. Proximal intestinal mucosa showed much congestion and many punctate hemorrhages.

These animals were operated on in a practically identical manner at varying periods after eating, and none was fed anything after operation. The one which lived the longest, one hundred and sixty hours, was fed twenty hours before operation, and the one that died in ten hours was fed the last time twenty-seven hours before operation. One dog which ate a full meal only six hours before operation lived one hundred and sixteen hours, while one that had fasted for seventy-two hours lived fifty-five hours after operation. The dogs had all the water they would take, both before and after the operation.

There is seen to be a complete lack of correspondence between the state of digestion at the time the obstruction is produced on the one hand, and the severity of the symptoms and length of life afterward. The same thing was noted in all subsequent experiments. It seems, therefore, reasonably certain that alterations of food residues in the tract above the obstruction has little to do with causing death.

The condition of the dogs after operation can be very briefly summarized. They showed a remarkable absence of active symptoms. Recovery from operation was usually prompt and complete, and on the next day they seemed well, but less lively than before. There was a slight rise in temperature Water was drunk freely, and promptly vomited, washing

out bile and mucus. Emaciation was marked. After a varying time the animal began to grow weak, but there was a lack of many of the symptoms we are accustomed to see in the human subject. Death seemed to result from general weakness and prostration. There was no abdominal distention, and no sign that cramps or colic were present.

An autopsy was done in every case, and in none was there any gross alteration in the organs that accounted for death. The peritoneum was not inflamed. The stomach and the intestine above the section were sometimes but not always markedly distended. They usually contained only a very small amount of bile and mucus. The mucosa showed a severe congestion. In one or two cases there was a superficial ulceration in the intestinal mucosa. We were struck with the fact that the most severe changes of this character were present in the dogs living the shortest time. We failed, however, to give this observation its full value at that time. The gall-bladder was not distended. The kidneys and liver showed congestion, and the former evidence of degeneration.

From these cases it was determined that animals with a high obstruction might live from five to seven days; that the length of life was not dependent on the presence of food residue in the tract above the obstruction, and that autopsy gave no gross evidence of the cause of death. A complete microscopic study of these cases was not made. Where sections were examined, however, the same lesions were found in the intestinal mucosa, the kidney and the liver, as those to be described in the second group of cases.

We next turned our attention to the production of an obstruction which should not be complicated by any operative damage to the intestinal wall. This was considered advisable because there seemed to be a direct relation between the severity of this damage on the one hand, and the severity of the symptoms and the early death of the dogs on the other.

A special clamp was devised which proved to be efficient in causing a complete obstruction, without opening the intestine or damaging the blood-supply. The clamp consisted of two rubber-covered aluminum plates, about 1 cm. in width and 4 cm. long. Over each end a second covering of rubber tubing was placed. One plate was then passed through a slit in the mesentery, with the bowel passing over it. The second plate was placed across the bowel at this point, and the two plates bound together near their ends. The interval left between them because of the rubber coverings over the ends allowed sufficient room for the intestine, tightly compressed, but not strangulated, to pass through. By bending the plates in or out the degree of obstruction was accurately regulated. We found by experience that the proper degree was obtained when the thin handle of a scalpel could just be passed within the bite of the plates, but not through it.

EXPERIMENTS WITH CLAMP OBSTRUCTION

EXPERIMENT 19.—Male. Weight 13.5 kg. He had been in laboratory seventy-two hours without feeding, and had been given only sterile water to drink. This was done with the idea of getting the stomach and upper intestine as nearly free from bacteria as possible. Cushing and Livingood[15] have shown that the number present can be reduced very markedly in this way. The clamp was placed 18 cm. below the pylorus. The dog remained in excellent condition for five days following the operation. The temperature ranged from 37.5 to 38.5 C. and the pulse from 132 to 150. He drank freely of sterile water, which he vomited soon afterward, washing out bile and mucus with it. He passed small quantities of urine, which contained traces of bile and albumin. During this period he lost 1.8 kg. He showed no active symptoms of the disease, and was in no apparent pain. The abdomen was not tender to palpation, nor was it distended. At operation the circumference of the abdomen was 47 cm. and on the fifth day only 43 cm. After the fifth day he began to show marked weakness and a more rapid loss of weight. The pulse and temperature remained about the same, and no new active symptoms developed. On the seventh day he was markedly weak and walked with difficulty. Emaciation was marked, there having been a loss equal to 19 per cent. of his body weight. He was killed with chloroform one hundred and seventy-two hours after the operation and the autopsy, with bacterial examination, immediately done.

The obstruction was tested under water-pressure, and was found complete, no water passing through the clamped point. Coils of intestine and omentum were matted around the clamp. When these were separated a small local abscess of green odorless pus was opend. The peritoneal coat of the intestine, at this point, was eroded by pressure, but no serious damage was done to the rest of the wall. The general peritoneal cavity was entirely free from evidence of inflammation. The liver and kidneys showed a marked congestion. There was a marked dilatation of the stomach and upper intestinal segment, and both contained water with bile and mucus. Their mucosae were markedly congested.

Microscopical Examination.—The intestine proximal to the clamp shows intense congestion and considerable exudative inflammation of the subserous tissue. The submucosa shows hemorrhagic and leukocytic infiltration. There is an acute hyperplasia of the lymph follicles. Distal to the clamp the intestine is normal. The kidneys show intense capillary congestion, especially of the glomeruli. The epithelium is not well preserved.

The spleen shows congestion.

The pancreas is normal.

The liver sections were unsatisfactory.

The cultures from the abscess around the clamp showed a pure culture of a Gram-negative bacillus, having the characteristics of colon bacillus. Cultures taken from the inside of the intestine, just above the clamp, showed the same bacterial growth, and in addition, a Gram-positive coccus. Our effort to sterilize the upper bowel was not successful, and the local abscess probably resulted from the passage of organisms outward through the intestinal wall, where the peritoneal coat was damaged by the clamp pressure. Other organs were sterile (see table).

EXPERIMENT 20.—Dog. Weight 9.5 kg. No food for one hundred hours. Sterile water during this time. Repetition of Experiment 19. Postoperative symptoms the same. Lived ninety hours. Autopsy (five hours post mortem, during which time the body was on ice) showed no peritonitis. Obstruction complete. Stomach a little dilated. Bowel above clamp twice the normal size. In the intestine, distal to the clamp, were seen many hemorrhagic points.

15. Cushing and Livingood: Johns Hopkins Hosp. Rep., 1900, ix, 543.

Microscopic Examination.—Intestine proximal to clamp shows fibrinous peritonitis, marked congestion of all coats, and exfoliation of lining mucosal cells. Stomach normal.

Liver: Congestion. Moderate granular and fatty degeneration.

Kidney: Moderate congestion. Tubules show granular coagulum. Cells swollen and show marked granular degeneration. Some cells show partial necrosis.

Cultures from femoral blood, peritoneum and liver show neither aerobic nor anaerobic growth.

EXPERIMENT 21.—Puppy. Weight 8 kg. Fed four hours before operation. Clamp applied 16 cm. below pylorus. The stomach contained food and the lymphatics and vessels were distended from full digestion. The postoperative course was the same as in the others. Pulse, 190. Temperature, 39 C. He died fifty-eight hours after operation. Autopsy, eight hours post mortem, the body being on ice. No peritonitis. Moderate distention stomach and duodenum. Few drops of purulent-looking fluid around clamp, buried in adhesions. The obstruction was complete. The gastric and intestinal mucosa, proximal to the clamp, showed very little change. Distal to the clamp there were areas which resembled submucous hemorrhages in the intestinal wall. The other abdominal organs appeared normal.

Microscopic Examination.—Proximal intestine not examined. Distal intestinal mucosa shows hyperplasia of lymph follicle, but no hemorrhages.

Liver: Marked congestion.

Kidney: Congested. Tubule cells swollen, and in places intensely degenerated. Apparent necrosis present in some foci of the tubules.

Spleen and pancreas much congested.

Bacteriology.—Cultures from femoral blood, peritoneum and liver all sterile, both aerobic and anaerobic, with exception of one colony on one peritoneal aerobic slant agar tube. This showed pure growth of a Gram-positive coccus and probably was an air contamination, one of the three found in the whole series.

EXPERIMENT 22.—Bitch. Weight 10.25 kg. Fed eighteen hours before operation. Clamp applied 12 cm. below pylorus. This animal was killed on the twenty-first day, up to which time she had showed no symptoms of suffering or of disease, except for loss of weight to 7.4 kg. During this time she had eaten nothing. She drank water freely, however, for the first three days. After this she received 150 to 250 c.c. of normal saline solution subcutaneously, and then she refused water to drink. She only occasionally vomited. The temperature ranged between 37 and 38 C. The pulse for the first four days was about 130. It then became slower and during the last two weeks was between 70 and 100. Urine was passed freely and contained bile and albumin in small quantities. Several blood-cell counts were made during the later days of the experiment and the red cells numbered 5,240,000 and 7,000,000, nearly all counts being slightly over 5,000,000. Killed with ether on the twenty-first day while still in good health. Autopsy. No peritonitis. Clamp allowed minimal drainage into intestine below (compare Experiment 27). Stomach and duodenum were somewhat dilated and the intestine distal to clamp was contracted, showing very little flow into it. It contained bile, however, and under pressure water was forced through the clamp into the bowel below. The mucosa of intestine and stomach appeared normal. The kidneys and liver showed congestion.

Microscopic Examination.—Stomach and intestine not examined.

Kidneys: Tubules filled with granular coagulum. Cells show swelling and granular degeneration.

Liver: Intensely congested.

Spleen: Congested.

Pancreas: Normal.

Bacteriology.—Neither aerobic nor anaerobic growth on tubes from femoral blood, liver or spleen.

EXPERIMENT 23.—Dog. Weight 11 kg. Clamp applied 20 cm. below pylorus. He was killed on the tenth day. His symptoms were the same as in cases above. He received saline subcutaneously with apparent benefit. Pulse, 132 to 150. Temperature 37.9 to 39.2 C. Weight at death 8.5 kg. Urine contained albumin and bile. An extensive noma developed on face which accounted for high temperature. Autopsy immediately after death by cyanid. Obstruction complete. Stomach and proximal intestine much dilated. They contained about 300 c.c. of bile, mucus and water. Distal intestine collapsed. The specimen was preserved intact so the mucosa was not seen. No peritonitis. Liver and kidneys much congested. The latter showed acute degeneration, the markings being indistinct and the papillae pale.

Microscopic Examination.—Kidneys showed granular degeneration, in places very marked.

Liver: Intensely congested. Considerable fatty and granular degeneration.

Spleen and pancreas show congestion.

Bacteriology.—Aerobic and anaerobic cultures from femoral blood and liver were all without growth.

EXPERIMENT 24.—Small bitch. Weight 6 kg. Fed seventy-two hours before operation. Clamp applied 16 cm. below pylorus. Lived 205 hours—a little less than nine days—with symptoms as in other cases. Pulse, 120 to 180. Temperature average, 39 C. Received saline injections throughout. Red blood-cells increased from 5,000,000 before operation to 6,720,000 on eighth day, showing some loss of water. Weight 4.9 kg. Died during night. Autopsy at 10 in morning. Obstruction complete as tested by absence of bile in distal intestine, although under pressure water could be forced beyond the clamp. No peritonitis. Around the clamp in a mass of omentum there was a collection of dirty yellowish fluid. The intestinal wall showed some damage within the bite of the clamp (see microscopic examination). The stomach and proximal intestine were moderately dilated, and contained a small amount of bile and mucus. The mucous membrane of the whole tract appeared normal to gross examination, as did the kidneys and liver.

Microscopic examination.—The intestine within the clamp showed an advanced purulent peritonitis, destroying longitudinal muscular coat. Congestion of mucosa. Exfoliation of epithelium.

Kidney: Congested. Tubules filled with granular coagulum. Cells swollen and show marked granular degeneration; in places approaching necrosis.

Liver: Section lost.

Pancreas: Marked congestion.

Bacteriology.—Aerobic and anaerobic cultures from femoral blood, peritoneum and liver showed no growth. A Gram-positive coccus was obtained in pure culture from the abscess around the clamp in all tubes both aerobic and anaerobic. This was undoubtedly an infection passing through the intestinal wall where the clamp damaged it, since the same organism was isolated from the intestine.

EXPERIMENT 25.—Dog. Weight 6 kg. Fed twenty-seven hours before operation. Clamp applied 16 cm. below the pylorus. He took ether badly and made a poor recovery. He was not given the hypodermoclysis of normal saline solution. Condition seemed good the next day. Died that night. Autopsy not done till day following, about thirty hours post mortem. Obstruction complete under water-pressure. No peritonitis. Stomach and proximal intestine somewhat distended. Distal gut collapsed. Mucosa of stomach and proximal gut markedly congested. Liver, spleen, pancreas and kidneys show congestion.

Microscopic Examination.—Stomach congested. Proximal intestinal mucosa shows intense congestion. Marked round-cell infiltration of tips of villi and exfoliation of cells.

Liver: Intense congestion. Considerable fatty and granular degeneration.

Kidney: Not examined.

No bacteriological examinations made because of delayed autopsy.

EXPERIMENT 26.—Bitch. Weight 7.2 kg. Exact duplicate in every way of Experiment 25, except that she received the saline injections daily, the first one of 150 c.c. being given at the close of the operation. She lived ten days, and showed the same symptoms as did other dogs described. Killed with ether and autopsied immediately. Obstruction complete against a column of water 90 cm. high. No peritonitis. The stomach and proximal intestine were dilated to about three times normal. The mucosa of stomach and proximal intestine were congested. The liver, kidneys and spleen were congested.

Microscopic Examination.—Proximal intestinal mucosa shows intense congestion and exfoliation of living cells.

Kidney: Shows congestion. Tubules contain coagulum and occasional blood-cells and show granular degeneration, in places very marked.

Liver shows intense congestion, atrophy of liver cords about distended capillaries and moderate granular degeneration.

Spleen shows much hypertrophy of the follicles.

Pancreas normal.

Cultures from femoral blood, spleen, liver and peritoneum were taken. These were all without growth after four days, under both aerobic and anaerobic conditions, except one aerobic tube (four were taken) from the liver. This showed one colony of Gram-positive cocci growing well to one side of the stroke of the loop.

EXPERIMENT 27.—Bitch. Weight 11.5 kg. Fed twenty-eight hours before operation. Clamp applied about 15 cm. below the pylorus. Lived twenty-four days with only moderate development of symptoms during first week. Then behaved like a normal dog. Reoperated on July 20. The clamp was not sufficiently tight to make a complete obstruction (compare with Experiment 22). It was tightened and the next day it had cut its way through the intestinal wall and the dog died of a peritonitis.

EXPERIMENT 28.—Dog. Weight 6.5 kg. Fed twenty-eight hours before operation. Clamp applied 11 cm. below pylorus. Postoperative course as in other cases. Died in fifty-six hours and body immediately placed on ice. Autopsy ten hours later. Obstruction complete. No peritonitis. Local infection around the clamp. Stomach and proximal intestine only slightly distended and contained a small amount of bile-stained fluid. The distal intestine was totally empty. Two superficial ulcers were seen in the mucosa of proximal intestine. The liver, kidneys, spleen and pancreas appeared normal.

Microscopic Examination.—Intestine not examined.

Kidney: Tubules swollen and show granular degeneration, very marked in places.

Liver: Intensely congested.

Bacteriology.—Cultures from the femoral blood, the peritoneum, the liver, the spleen and the local collection of fluid around the clamp all were without growth under aerobic and anaerobic conditions.

EXPERIMENT 29.—Dog. Weight 8 kg. Fed thirty hours before operation. Clamp applied 11.5 cm. below pylorus. Postoperative course same as in other cases. Received about one-fortieth of body-weight of normal saline subcutaneously daily. He remained in excellent condition for eight days, during which time he lost a little over 2 kg. in weight. He drank water each day, which was always vomited. Pulse from 130 to 174. Temperature, 38.5 to 40 C. On the ninth day he was etherized and the abdomen opened. The obstruction was complete. The stomach and proximal intestine were much distended and filled with a greenish fluid of the thickness of pea soup. There was no peritonitis. The splanchnic vessels were not much dilated, except those above the occlusion. The spleen was not enlarged. The liver and kidneys did not show much congestion. Sections were taken from the stomach, duodenum, distal intestine, liver, spleen and kidney while the dog was still under ether, after which he was killed.

Microscopic Examination.—The stomach is normal.

The proximal intestine shows marked congestion and an edema in the submucosa. The lesions here were not advanced, because the specimens were taken some hours before the animal would have died from the disease. The distal intestine is normal.

The liver is congested. The cells show granular degeneration and areas of necrosis are present.

The kidney is congested. Tubules contain granular coagula. The cells show granular degeneration.

The preceding series includes experiments on eleven animals which may be summarized. In two a complete obstruction was not obtained because the clamp was not sufficiently tight. The two animals were killed after twenty-one and twenty-four days, respectively, while each was in good condition. They had lost considerable weight, and one of them was weak, but otherwise they acted like normal dogs.

The remaining nine dogs all showed a similar condition while living, and autopsy yielded the same findings in the organs. In some dogs, particularly those which lived the longest, the dilatation of the stomach and the duodenum was very marked.

Four dogs in this series were given sterile water by mouth only, and this they promptly vomited. They were, therefore, practically getting neither nourishment nor water, for if what they drank was not all vomited, very little absorption could take place from the stomach and duodenum. Beginning with the fourth dog, each dog received from 150 to 300 c.c. sterile normal saline solution, subcutaneously, per day. This was absorbed. In one case this was not given till the third day. This animal had then begun to act sick, but showed a marked improvement after the administration of the hypodermoclysis. In general, the dogs which received this saline lived longer than the others. They sometimes drank sterile water in addition, but this they always vomited in a very short time. In many cases they refused to drink more than a few cubic centimeters. The three dogs which lived the longest were all given the saline subcutaneously, and there seems no doubt that this procedure was very beneficial.

We attempted, by a count of the red blood-cells, to determine whether the dogs not receiving it suffered from dehydration, with a consequent increase in the number of red cells per cubic centimeter. There was not a sufficient variation from the normal to throw any light on this point, the number ranging around 6,000,000 both before and after the saline injections. The longest postoperative life was ten days (two dogs); a third dog lived just under nine days; the shortest period was thirty-six hours in a small fox terrier which never completely recovered from the operation. The average length of life was one hundred and thirty-nine hours in this series, as against sixty-seven hours in the series in which the obstruction was produced by sectioning the intestine and inverting the ends. It was only in this series that the saline injections were used.

It was, moreover, remarked that the second series, in which no damage to the intestinal wall was produced, was much nearer the normal throughout the experiment than was the first series, in which the intestine was sectioned. So far as we know, no other investigator has paid the same attention to producing an obstruction without any damage to the intestinal wall. There is evidence that the method is of advantage in excluding the complications which may arise from such damage. Clinically, we know that when a strangulation, however limited in extent, is superimposed on an obstruction, the course of the disease becomes much more acute. Medowoy,[16] Kocher[17] and others emphasize this point.

Murphy and Vincent,[12] in their experiments, worked particularly on this phase of the question. In a simple obstruction in cats they found the intestinal wall very little damaged in four to six hours. When, however, the circulation was disturbed, particularly a venous stasis produced, a very severe change was present at this time. They conclude that "interference with the circulation of the obstructed intestine is the vital factor in the production of the symptoms of ileus."

When the intestine is sectioned, and the proximal end sufficiently inverted to prevent the stomach and duodenal contents being forced through it, a local strangulation takes place. At autopsy there was always found in these cases a local damage to the intestinal wall at the inverted point, small in extent, to be sure, but nevertheless sufficient to be the possible site of the absorption of a considerable amount of poison. Often, too, a very considerable intussusception of the invaginated stump was present. This mechanical damage, of course, is not an essential in producing death, because it was not present in the second series, in which the animals died. We feel, however, that it has an important bearing on the problem, as we shall later more fully explain.

We desire to call particular attention to the microscopic findings in these cases. The intestinal mucosa, proximal to the obstruction, shows a marked congestion. Marked round-celled infiltration is present, and exfoliation of the lining cells. Hemorrhages are often seen. There is present an acute hyperplasia of the lymph follicles. The submucosa is edematous. Distal to the clamp there is no change, or only a very slight change, from the normal. The gastric mucosa shows only a congestion. The kidneys are markedly congested. The tubules contain granular coagula. The tubule cells are swollen and the seat of granular degeneration; not infrequently necrotic areas are seen.

The liver is congested and the cells show granular and fatty degeneration. Necrotic areas may be present.

16. Medowoy: Deutsch. Ztschr. f. Chir., 1910, cv, 1.
17. Kocher: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1898-99, iv, No. 2, p. 195.

The spleen shows marked congestion, and some hyperplasia of the lymph follicles.

The pancreas shows only congestion.

The changes in the intestinal mucosa are worthy of careful study. They may result from two causative factors. The marked distention and stretching of the intestinal wall may produce a circulatory disturbance or possibly an actual trauma to the mucosa. The stagnated secretions may be sufficiently irritating to damage the mucosal cells chemically. In either case there would develop a condition of lessened resistance, and the intestinal bacteria would be free to attack the mucosa and bring about the changes seen. This having occurred, the changes in the other organs may be conceived as having been caused by the entrance into the blood of poisonous materials which were produced by this bacterial activity. Sufficient evidence that such an explanation is correct is found in the fact, already pointed out, that there is an apparent parallel between the extent of the changes in the mucosa and the severity of the symptoms.

The evil effects of hyperdistention to the intestinal circulation has been experimentally shown by Van Zwalenburg.[18] By means of illumination in the intestinal lumen he watched the circulation when different degrees of pressure were exerted inside the bowel. At a pressure of 30 mm. mercury it was noted that some capillary streams were arrested, while at 60 mm. small veins became blocked. Above this the circulation showed increasing disturbance. If a continuous pressure of from 80 to 90 mm. were continued for an hour, the apparent number of veins seen on the serous surface was greatly increased. Their caliber was also greater than normal. This observation would seem to indicate that the effect of the long-continued distention in a blocked bowel must seriously damage the integrity of the mucosal circulation and lead to the lesions observed in our experiments and recorded above.

The influence of the stagnated secretions above the obstruction may be considered as important in giving rise to poisonous materials which could enter the circulation through the damaged intestinal wall. In order to study this phase of the question, we turned our attention to an elimination of the bile, pancreatic and duodenal secretions as a source of the poisons. To this end, in five dogs the following operation was done: The pylorus was divided and the two cut ends closed by inversion. The intestine was divided just distal to the duodenum, and the proximal end implanted in the jejunum about 30 cm. lower down. The distal end was implanted into the stomach. By this means the stomach emptied into the intestine just below the duodenum, and the bile, pancreatic and duodenal secretions passed into the jejunum about 30 cm. distal to this. Our intention was to cause an obstruction with the clamp, just orally

18. Van Zwalenburg: Ann. Surg., November, 1907, xlvi, 780.

to the new duodenal outlet when the animals should have recovered from the anastomoses, thus having an obstruction of the stomach and about 30 cm. of the intestine but not of the duct-bearing portion of the tract. For a few days following the operation these dogs did well, fully regained their appetites and seemed to be in good health. After about a week, however, they began to act sick. They did not eat well, and sometimes vomited. They lost weight rapidly. Some of them developed diarrhea with bloody-appearing stools. Four of them died in about four weeks with evidence of malnutrition. The autopsy showed, in two of these, that a very extensive damage to the intestine between the stomach and duodenal anastomosis was present. Ulcers were seen which were about to perforate. The stomach and this part of the intestine contained partially digested blood.

The fifth dog seemed to be in fair condition after two weeks, and on the nineteenth day the abdomen was opened under ether anesthesia. The anastomoses were satisfactory and no evidence of ulceration was present from the peritoneal surface. The occlusion clamp was applied just above the anastomosis of the duodenum with the jejunum. The following day the dog was in good condition; he had not vomited. He was given saline subcutaneously. He died during that night and autopsy was done in the morning. The anastomoses were satisfactory. A general peritonitis was present from a perforation within the bite of the clamp. The stomach and intestine anastomosed to it were distended with a dirty brownish fluid. When they were opened the gastric mucosa was seen to be much congested; the mucosa of the jejunal segment was markedly congested and contained many hemorrhagic areas. The intestine below the duodenal anastomosis was entirely normal in appearance. The effort to produce an obstruction in which the duodenal contents should not be stagnated had not been successful. But the symptoms developing in these dogs and the findings in the intestinal mucosa added emphasis to the relations existing between these two factors. In many ways these dogs behaved like the ones with an obstruction, the symptoms and the course of the disease being, however, much less acute. The microscopic examination of the intestinal mucosa, the kidneys and the liver, showed, too, the same lesions as found in the simple obstruction.

The fact that such damage to the intestinal wall, in so far as it occurs in intestinal obstruction, is the cause of serious symptoms, and, if extensive enough, may even cause death, seemed to be well demonstrated. That it is the sole factor, however, was not proved, and as stated above, our attention had been arrested by the benefit derived from the administration of salt solution under the skin. It was conceivable that this benefit arose from diluting and eliminating the poisons absorbed through the damaged intestine. Such a supposition, however, had no support in any work done, and accordingly a more detailed study of this question was

undertaken, and for this purpose Dr. Benedict, of the department of chemistry, kindly offered to make some studies in metabolism for us. To him we are indebted for a most important discovery which, we believe, throws much light on the question under consideration, if it does not completely solve the problem of the cause of death from uncomplicated intestinal obstruction.

EXPERIMENT 1.—The first dog under his observation was a female in good condition, weighing 12.1 kg. The clamp was applied under ether narcosis on the lower part of the duodenum, March 4, 1912. Table 3 gives the results found during the three and a half days that she lived.

TABLE 3.—RESULTS OBTAINED AFTER CONSTRICTION OF THE LOWER PART OF THE DUODENUM FOR THREE AND ONE-HALF DAYS

Date	Weight, kg	Urine, c.c.	Vomitus, c.c.	Combined Fluid Output, c.c.	Total Nitrogen, gm.	Remarks
3/4/12	12.10	..	....	....	....	Clamp applied to lower duodenum.
3/5/12	11.28	26	720	746	....	Received 100 c.c. of a 0.9 per cent. saline solution.
3/6/12	10.36	15	500	315	10.334	Received 100 c.c. of a 0.9 per cent. saline solution.
3/7/12	9.86	86	510	596	....	Received 100 c.c. of a 0.9 per cent. saline solution.
3/8/12	....	..	....	....	....	Died.
Totals,	....	127	1,730	1,857	....	

From these figures it is seen that she lost 2.2 kg. in weight. Her output of fluids, including the urine and vomitus, but not counting the water of expiration, was 1,857 c.c., to offset which she had only received 300 c.c. subcutaneously. The total nitrogen output in two days was 10.34 gm., which equals, approximately, 0.5 gm. per kilo per day, which is two or three times the normal output. A sample volume of urine of 127 c.c. contained 3.574 gm. albumin. The creatin excreted in this equaled 0.638 gm., which was more than double the amount of creatinin.

It was obvious from these figures that a very severe grade of tissue disintegration was taking place, and that a water loss averaging about 5 per cent. of the body weight was suffered each day. It at once became apparent that the amounts of water we had been giving were entirely inadequate to replace this great loss, and that probably death was much hastened by such a loss.

Accordingly, we made a study of three dogs in which the daily output was measured, and a quantity of normal saline about 100 c.c. in excess of this was injected under the skin. These dogs with the clamp applied on the lower duodenum were kept alive in this way for three weeks (one for twenty-six days), and during this time they remained in good condition. They were then etherized a second time, and the abdomen opened to see if the obstruction were complete. In every case it was found to be so. The stomach and the duodenum above the clamp were excessively dilated, and their contents could not be forced through the

clamp into the intestine below, which was found empty and much contracted. By varying the amount of water injected, we could accurately control the condition of the dogs. The complete study of one of the experiments is shown below.

TABLE 4.—RESULTS OBTAINED WITH CLAMP ON THE DUODENUM FOR THREE WEEKS AND INJECTIONS OF SODIUM CHLORID

Date	Weight, kg.	Urine, c.c.	Vomitus, c.c.	Combined Fluid Output, c.c.	Injection 0.9% NaCl. Sol., c.c.	Remarks
3/13/12	11.3	....	....	....	....	Clamp applied to lower duodenum.
3/14/12	....	120	420	540	250	
3/15/12	....	175	—	175	200	
3/16/12	....	160	600	760	200	
3/17/12	10.46	170	—	170	200	
3/18/12	10.84	135	—	135	500	
3/19/12	....	†	†	...	150	
3/20/12	....	130	850*	980	250	
3/21/12	9.64	130	—	130	550	
3/22/12	10.24	100	550*	650	850	
3/23/12	....	80	800*	880	500	
3/24/12	10.24	...	....	...	600	
3/25/12	9.68	70	1,180*	1,250	750	
3/26/12	....	†	†	...	800	
3/27/12	....	85	1,160*	1,245	800	
3/28/12	10.00	310	—	310	700	
3/29/12	....	562	150*	712	300	
3/30/12	....	390	—*	390	200	
3/31/12	....	150	200*	350	200	
4/1/12	....	55	80*	135	150	
4/2/12	8.9	55	—*	55	100	Is in excellent condition.
4/3/12	8.3	...	650	650	—	Obstruction found complete. Clamp removed. Recovery.
Total for 21 days.....		2,877	6,640	9,517	8,250	

* Obtained by stomach-tube. † Incomplete. — None.

EXPERIMENT 2.—A bitch weighing 11.3 kg. had the clamp applied under ether narcosis, on the lower duodenum, March 13. April 3, twenty-one days later, when in excellent condition, she was again etherized and the abdomen reopened. The obstruction was found to be complete. The clamp was removed and she is still alive. The total vomitus during the twenty-one days was 6,640 c.c. and she received to offset this 8,250 c.c. of saline solution. Much of the vomitus had to be obtained by the stomach-tube, as the stomach became dilated and would not empty itself. She lost 3 kg. in weight. The study of the urine gave most interesting results. During the first week it showed very marked abnormalities. The albumin content was as high as 4.5 gm. per day, and the total nitrogen, exclusive of this, was, on an average, 5 gm. The creatinin-creatin ratio was about 1 to 2 instead of 10 to 1, which is near the normal for a starving dog. The latter ratio was later established. During this week the vomitus was 1,870 c.c. in amount, which was 120 c.c. more than the amount of saline solution administered. The urine equaled 890 c.c., so that there was a loss of water equal to one-tenth of the body-weight. The condition of the animal was only fair during this week. After the seventh day the saline was increased to 550 c.c. daily and maintained there, or higher, for several days. The abnormalities in the urine entirely disappeared, and it became the urine of a dog under normal starvation, except that it contained bile throughout. There was coincident improvement in her condition. Later, on the sixteenth day when vomiting had ceased and the stomach-tube recovered very small amounts, the saline was again decreased to 200 c.c. or less; the dog remained well, and the urine continued normal.

EXPERIMENT 3.—A small bitch weighing 5.88 kg. had the clamp applied under ether narcosis to the lower duodenum, April 8, 1912. She received the normal

saline solution subcutaneously, every day, the first injection of 300 c.c. being given immediately after the operation. The quantity was repeated on the two following days, and then for sixteen days she received 400 c.c. daily, and for two additional days 300 c.c. each. The total saline administered during the continuance of the experiment for twenty-one days was 7,900 c.c. an average of 376 c.c. per day, which was equal to about 8 per cent. of her average body-weight. During this time the total amount of fluids excreted in the urine and the vomitus was 6,354 c.c. Much of the vomitus had to be excreted by means of the stomach-tube because the stomach became atonic and vomiting ceased even though the organ was full. Her loss in weight was 1.4 kg. Throughout the entire experiment she remained in a condition of apparent perfect health. She was always playful and lively. On the twenty-first day she was etherized and the abdomen was reopened. The clamp was found *in situ* and the obstruction was complete. Four hundred and fifty c.c. of water were put in the stomach through the tube. This readily passed through the pylorus and down the duodenum to the clamp, where it was completely stopped. It was impossible to force any beyond the clamp by direct pressure on the duodenal wall. The clamp was removed and a small perforation, which was completely closed by the rubber covering of the plate and by adhesions, was found in the bite of the clamp. There was no peritonitis.

EXPERIMENT 4.—A bitch weighing 12.2 kg. had the clamp applied under ether narcosis to the lower duodenum, April 19, 1912. During the following six days she received 1,500 c.c. of normal saline solution subcutaneously, and excreted in the form of vomitus and urine 1,020 c.c. On the seventh day she did not vomit, and the stomach-tube recovered nothing. We suspected that the clamp was not tight enough to produce a complete obstruction, and she was accordingly given 500 c.c. of water through the stomach-tube and a quantity of meat which she ate greedily. Following this she again vomited, and in four days put out 2,150 c.c. in which the digested meat was present. The injections were then increased, quantities varying from 700 to 500 c.c. being given daily. The vomiting ceased and a quantity of urine was passed, slightly less than the saline injected, the total for seven days being, saline 4,000 c.c., urine 3,200 c.c. The saline was again decreased, and the urine fell, but no vomiting recurred. She was etherized May 16, twenty-six days after the obstruction, and the abdomen was reopened. The obstruction was found to be complete. The stomach and duodenum were found to be enormously dilated, having a capacity of 1,000 c.c., while the intestine below the clamp was contracted and empty, not showing any trace of bile. No detailed study of the urine was made in this or the preceding case.

It will be noted that in many instances there was no vomiting, but the stomach-tube recovered large quantities. This must be taken into account in determining the amount of saline to be administered, because whether vomited or retained in the stomach, it means a loss to the animal. These three dogs were under identical conditions with the nine previously reported, except that they received amounts of normal saline solution about 100 c.c. in excess of the daily output of vomitus, often reaching 500 to 700 c.c. per day, while the animals in the former series received only 150 or 200 c.c. per day. No dog in the first series lived longer than ten days. All dogs in the later number were alive and in good condition at the end of three weeks, and apparently would have succumbed only to starvation if the experiment had been continued.

In order to be certain that no leakage took place, an obstruction was produced in two dogs by dividing the lower duodenum and closing the

ends by inversion. The result was the same as in the dogs having the clamp applied. Care must be taken, however, to minimize the amount of damage done to the intestinal wall in this operation, otherwise the animal is poisoned from this source and may die.

From these results it is plain that the loss of fluid from the organism is the disturbing influence and is the important factor in explaining the symptoms and death following uncomplicated high intestinal obstruction in dogs. Death is caused by the loss of water which the tissues sustain from the excessive amounts vomited, and death can be prevented if this water be replaced by cutaneous injections of normal salt solution.

The source of such enormous amounts of vomitus is a matter of interest. Dr. Benedict's study of the vomitus from the experiment detailed under Table 4 showed it to be strongly acid, and to have a specific gravity of 1.007. The total amount contained 16.72 gm. of chlorin, which, computed as sodium chlorid, equals 27.65 gm. Bile was always present. The low specific gravity indicates that it was largely water. These findings show, as would be expected, that the vomitus is a diluted mixture of bile and gastric secretion. The part played by the pancreas and duodenum is unknown. In all the cases the vomitus was more abundant during the early part of the experiment than it was later, and toward the end it often almost entirely disappeared. The following considerations offer an explanation of this unexpected fact. The dogs were all in good condition when operated on, and presumably the liver and other glands were actively secreting. The products of these glands, bile, pancreatic juice and duodenal secretion, found no outlet beyond the duodenum, and hence were forced back into the stomach. Here they acted as an irritant to the gastric mucosa, and as a result a secretion of gastric juice and an excretion of water took place to the enormous amounts found. As the experiment proceeded the dog gradually developed the condition of a starving animal in which all the alimentary glands become inactive, the bile in particular being very small in amount. The disturbing influence of these retained secretions being absent, the vomiting gradually grows less, and finally almost ceases. The small amount of bile secreted is taken care of in the duodenum and stomach, being either digested or absorbed. That some is absorbed is shown by the fact that the urine contains bile throughout the experiment. The bile alone, however, is not the offending factor, because in cases in which the common bile-duct was tied and an anastomosis made between the gall-bladder and the intestine below the clamp, large amounts of vomitus appeared, though not equaling those above given.

The results of all our work demonstrated, therefore, that there are two important factors concerned in producing the symptoms and causing death in a high intestinal obstruction in dogs.

First, the loss of water from the tissues caused by the excessive drainage into the intestinal lumen followed by vomiting. This factor is entirely under therapeutic control. It can be rendered harmless by the subcutaneous administration of normal saline solution.

Second, the absorption of poisonous materials from the intestinal wall, when, by reason of overdistention and chemical irritation the mucosa is damaged and bacteria set up an active inflammation in it, and possibly the other intestinal coats. This factor is not appreciably influenced by the administration of the salt solution.

REVIEW OF THE WORK OF WHIPPLE, STONE AND BERNHEIM

We have referred above to the work done by Whipple, Stone and Bernheim, which appeared[3] after preliminary reports of our work had been published,[19, 20] setting forth the conclusions just given. As their conclusions differ in important particulars from ours, it seems advisable to compare the two, and if possible reconcile the facts as a basis for the correct conclusion. In doing this we shall quote freely from an article already published,[21] in which this phase of the subject was treated.

Whipple, Stone and Bernheim,[22] studied the question by producing a closed loop of the duodenum, beginning just below the pancreatic ducts and ending beyond the suspensory ligament. This portion was isolated from the remainder of the intestinal tract by tying a heavy ligature tightly at these two points, and burying it under an inverted layer of the peritoneum. A gastro-enterostomy was then done. Dogs so treated died in from thirty-six to seventy-two hours. The authors inferred from this that there developed in the closed loop a poisonous material, the absorption of which caused death. Since the closed loop contained neither gastric secretion, bile nor pancreatic juice, the conclusion seemed inevitable that some alteration in the loop function was responsible for death.

A series of careful and well-controlled eperiments was then undertaken to study the nature, and if possible, to determine the source of any poison. The method employed was to produce the isolated loop, collect the contents, sterilize them by heating to 60 C., filtering them, and then, after autolysis, to inject them into a healthy dog, intravenously, subcutaneously or peritoneally. It was demonstrated in this way that dogs so injected died promptly. Further, the authors were unable to isolate any poisonous material from the normal intestine by any method of treatment; and if the mucosa of the loop were first destroyed by a

19. Hartwell and Hoguet: Am. Jour. Med. Sc., 1912, cxliii, 357.
20. Hartwell and Hoguet: Experimental Intestinal Obstruction in Dogs, with Especial Reference to the Cause of Death and the Treatment by Large Amounts of Normal Saline Solution, Jour. Am. Med. Assn., 1912, lix, 82.
21. Hantwell, John A.: Jour. Exper. Med., 1913, xviii, No. 2, p. 139.
22. Whipple, Stone and Bernheim: Jour. Exper. Med., 1913, xvii, 286.

fluorid, no poisons appeared. Lastly, some dogs died within a few days when the loop was drained externally, there being, according to the authors, an absorption directly from the mucosa, and not from the lumen.

The authors summarize, in part, as follows:

> There seems, therefore, no escape from the conclusion that a poisonous substance is formed in this closed duodenal loop which is absorbed from it and causes intoxication and death. Injection of this toxic substance into a normal dog gives intoxication and a reaction more intense than, but similar to, that developing in a closed loop dog (p. 306).
>
> The mucosa of closed or drained duodenal loops contains a toxic substance quite similar to the toxic material found in the lumen of the closed loops.
>
> Normal intestinal mucosa contains no toxic substance nor can it neutralize *in vitro* the toxic substance produced in the closed loops. Our experiments show that formation and absorption of a toxic substance may proceed in a loop whose blood-supply has in no way been disturbed, and whose mucosa is quite intact both in gross and microscopically (p. 323).

The authors apply their findings to the condition of simple intestinal obstruction, and state it as their belief that "the toxic material concerned is the same in both instances" (p. 321). They add: "Our experiments indicate that intoxication is the primary effect, and the loss of body fluids a secondary one, rather than the reverse, as is claimed by Hartwell and Hoguet."[20] In substance, they claim to have proved that a closed loop of intestine, without undergoing any morphological change, produces a toxic substance which is absorbed and causes death. Moreover, they infer that a similar condition exists in simple intestinal obstruction, and that here also death results from the absorption of this same poison.

We fail to find any evidence in their experiments, as published, to justify these claims. On the contrary, we find that the protocols definitely show a damage to the intestinal wall, and that the toxic substance they are dealing with arises from this source. In the absence of this damage no toxemia is present except that produced by the loss of water in the vomitus. We submit the following review of their protocols in support of this view:

> Dog S-34.—Moribund in twenty-seven hours. Killed with ether. Autopsy immediately. "The duodenal loop is of enormous size." "Purplish patches show through the transparent serosa." "The contents are blood-stained."
>
> *Microscopical Examination.*—"In places the wall of the duodenum shows necrosis, hemorrhage and invasion by leukocytes."
>
> Dog S-27.—Died in thirty-two hours.
>
> *Autopsy.*—"The duodenal loop is contracted and contains a little material. Its walls show a few areas of subserous hemorrhage, about 1 or 2 cm. in diameter. There is obvious necrosis associated with these areas, and through one of them rupture had taken place, with escape of duodenal loop fluid into the peritoneum." No microscopical examination is given.
>
> Dog S-20.—Death in forty-eight hours. One hundred c.c. of fluid are in the loop. "The closed loop mucosa is pinkish and a little swollen, but no ulcers are present." The proximal loop was not tight, and some fluid had probably regurgitated into the stomach.

Dog 56.—Death in fifty-five hours. Remained well for twenty-four hours.

Autopsy.—"The abdominal cavity is clear except for slight granular peritonitis about the loop and area of operation." "The small intestine below the loop contains blood-stained mucus, and its mucosa has a mottled appearance, with large areas of deep red color where the capillary injection is quite marked. The duodenal loop is rather flabby and not greatly distended. It contains about 50 c.c. of a thin, soup-like, faintly blood-tinged fluid. The loop mucosa is quite intact, but of a delicate pinkish tinge." No microscopical examination is given.

Dog S-53.—Death in three days. Seemed well for twenty-four hours.

Autopsy.—The condition of the loop mucosa is not given; it is merely stated that it is pink and intact. No microscopical examination is given.

Commenting on these four experiments the authors say:

The duodenal loop may show a normal intact mucosa in gross and microscopically, except for a little diffuse injection. Again, it may show necrosis, ulceration and hemorrhage, and the contained fluid may vary correspondingly in characteristics, but it always contains a toxic substance (p. 291).

In only one case, however, is a microscopical examination given, and then marked changes were found, while in the others evidence of change was seen grossly. The length of life seems directly to correspond to the lack of damage found in the mucosa, as demonstrated by the reported findings in Dog S-53, which lived for three days.

The following protocols give the conditions found in the duodenal loop, when the dog is killed at the end of twenty-four to twenty-eight hours. In one case, Dog S-4, the contents were blood-tinged, owing to inversion of the duodenal mucosa.

Dog S-4.—"The mucosa throughout the loop is intact, but slightly swollen and of a pinkish color."

Microscopical Examination.—"The tips of a few of the villi are slightly swollen and contain a few polymorphonuclear leukocytes. The epithelium is normal throughout."

Dog S-2.—In twenty-six hours the duodenal loop contained blood-tinged fluid; in places, on microscopical examination, "it shows some areas of necrosis with hemorrhage and invasion by pus cells, involving mucosa and submucosa."

Dog S-5.—The loop contained blood-tinged fluid.

Microscopical Examination.—"The duodenal loop is quite normal."

In two dogs, S-28 and S-29, the duodenal loop contents were not blood-tinged, and the mucosa, on gross examination, seemed normal, though no microscopical examination is given.

These dogs were all in fair condition when killed, being described as "dull and drowsy," "weak," "pulse strong," "somewhat dull but not very toxic." The incipient damage to the mucosa, as shown in these cases by the blood-tinged fluid and the microscopical examination, explains the cause of their condition. We cannot reconcile a normal mucosa with a blood-tinged content, as reported in Dog S-5.

Dogs S-12 and S-9.—Both dogs were killed after forty-four hours, and were apparently in fair condition, Dog S-9 showing a carotid pressure of 100 to 110 mm. of mercury, with almost normal pulsation. There were no visible changes in the loop mucosa, either on gross examination or microscopically.

Dog S-38.—The dog was killed after forty-eight hours, his condition being "somewhat toxic." "The blood-pressure was practically normal and the pulse regular." "The duodenal loop contains 20 to 30 c.c. of a strawberry-colored thick fluid. The loop shows a diffuse pinkish mucosa, no ecchymoses and no ulcers."

These dogs were killed after from forty-four to forty-eight hours, when in good condition, and no change was found in the mucosa. If the enclosed secretion from the mucosa itself is the active poison, the absorption of which, by the mucosa, causes death, why are the dogs not made more ill? We believe it is because in these individual cases the mucosa was not so severely damaged. If we compare them with Group 1, in which the dogs were allowed to die, and with the group immediately preceding, in which at the end of approximately twenty-four hours the dogs were apparently more toxic, and a definite change in the mucosa was found, we cannot escape the conclusion, from a careful analysis of the cases, that the severity of the toxic symptoms is in direct ratio to the damage done to the mucosa.

In their injection experiments (pp. 301-304) the authors prove beyond question that they have obtained from the contents of the closed loop a substance, which, after sterilization by heat (60 C. for thirty minutes) and filtering through a Gooch or other porcelain filter, is very poisonous, and will cause death in a few hours, if it be injected into the vein, the peritoneum or the subcutis of a dog.

Whipple, Stone and Bernheim find that a similar poison can be extracted from the mucosa (not the loop content) of a non-drained or a drained closed loop, injection of which into dogs produces severe toxic symptoms (pp. 310-311). They argue from this that the substance with which they are dealing is elaborated in the mucosa of the closed loop, and that some unknown force is at work which profoundly alters the activity of the epithelial cells. Yet they emphasize the fact that there is no demonstrable change from the normal. Where do they conceive this toxic substance to arise? They disprove Draper Maury's contention that it is a normal output of the mucosa, which is poisonous unless neutralized with fluids from other portions of the alimentary tract, by showing that duodenal loop fluid, digested with normal intestinal mucosa, loses none of its toxicity (p. 316). With a normally secreting mucous membrane, undergoing no change from the normal, it is difficult to conceive how there is rapidly formed a very toxic material which is directly absorbed from it into the general circulation, with resulting death. It seems inevitable that severe changes take place in the mucosa —they are usually demonstrable—and that the toxic material comes from this alteration in the mucosa. In all cases in which death occurs from the closed loop itself some evidence of damage to the mucosa is described, and the severity of the symptoms runs parallel to the severity of the demonstrable damage.

A careful study, therefore, of the findings of these authors makes us unwilling to accept their conclusion that a poisonous substance is poured out by a normal mucosa, or elaborated and directly absorbed by the normal cells, which substance produces the toxic symptoms in simple high intestinal obstruction. In discussing the fact that dogs with a simple obstruction may be kept alive at will by simply replacing with normal saline solution the fluid lost in the vomitus, Whipple, Stone and Bernheim say that the benefit derived "is due to dilution and elimination of the toxic material by this means, and the development of a gradually increasing immunity which can be shown to appear in dogs after intravenous injection of the toxic substance" (p. 321). Yet they report conclusive experiments showing that their toxic substance neither exists in the blood of a closed loop dog nor remains for two hours in the blood of a dog that has received a lethal dose intravenously (p. 309). It is difficult to comprehend, then, how saline solution given once a day is capable of washing out from the blood a substance which, within two or three hours, is "fixed by the cells or partly destroyed," and which, if not washed out, will inevitably cause death. If this were possible, one ought to find a cure for organic poisons by a hypodermoclysis, and, similarly, the closed-loop dogs ought to be saved by this means.

In commenting on the claim of Hartwell and Hoguet,[20] that this action of salt solution is a strong argument against an essential toxemia, and that the loss of water is the important factor, Whipple and his associates say: "One might argue in the same manner that the appearance of sugar in the urine is the cause of diabetes." The analogy is incorrect. Were it possible to alleviate diabetes by the subcutaneous injections of sugar, to the extent that obstruction may be alleviated by injections of saline solution, the analogy would be acceptable, the loss of sugar being the prime factor in one case, and the loss of water in the other.

Moreover, the most potent argument of Hartwell and Hoguet in this connection—a normal starving metabolism when saline is given, and a completely deranged one when it is withheld, as determined by Benedict—is entirely ignored by Whipple, Stone and Bernheim.

It is our contention that the experiments cited by these authors have no bearing on the subject of *uncomplicated* obstruction. They show, however, that a damaged intestine will produce a substance which is very poisonous if it gain access to the blood. This is exactly the same conclusion we arrived at by a different method of investigation and reasoning.

Still further to test the question, we produced the doubly closed loop in different parts of the bowel on a series of dogs.

The experiments with a doubly occluded loop of intestine, made either by section and inversion of the ends, or by the clamps, need not be detailed because they duplicated in every way the results obtained by Whipple,

Stone and Bernheim. It is worthy of note, however, that dogs with the double closure in the middle of the jejunum showed the same symptoms and died in the same way as those with a duodenal doubly closed loop. When the jejunal loop became distended with its secretions, and the mucosal circulation was damaged, the animals became sick and ultimately died. The course, however, was less acute. This proved that death is not due to any specific action of the duodenal mucosa, as Whipple, Stone and Bernheim assume. When, however, a double occlusion is produced in the lower ileum, or the cecal region, the animals are not sick, and they may live an indefinite time. Similar results have been obtained by von Baracz.[23] They are explained by the fact that this part of the intestine secretes very little fluid and consequently there is no distention, no damage to the mucosa, and no absorption of the poisons which higher up result from such a damage.

A series of experiments performed by us with the object of preventing such a damage even in the duodenum gave important results. We believed that if we could minimize the amount of fluids poured out into the duodenal loop and the operative damage to the bowel wall, we could longer retain an intact mucosa, and hence longer delay the fatal outcome. To accomplish this the operation was done in two stages. At the first stage the bowel was sectioned just below the pancreatic duct and the two ends closed by inversion, with as little operative damage as possible. The continuity of the alimentary canal was then reestablished by a lateral anastomosis between the upper duodenal segment and the beginning of the jejunum. In this way there existed a segment of the duodenum below the ducts which was closed at the upper end and drained by continuity directly into the jejunum. The animal was allowed to recover completely from this operation during a period of two weeks. He was then kept without food for four days, and the second stage operation performed. At this time the lower end of the duodenum was sectioned at the site of the entero-enterostomy and the two ends closed, the same care being taken to avoid needless damage to the mucosa in doing this. Thus, there resulted a doubly closed duodenal loop. Following this operation the animals received no food or water by mouth, but were given saline subcutaneously. This was done with the belief that absence of the stimulation from food would materially decrease the secretions in the closed loop, and thereby prevent its overdistention.

In a series of dogs we were able to get a positive result in two in that with a double occlusion of the duodenum without visible damage to the mucosa death did not result as it usually does in the one stage operation with its inevitable damage to the mucosa.

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23. Von Baracz: Arch. f. klin. Chir., 1899, lviii, 121.

Dog 107.—May 1, 1913: An entero-enterostomy was done between the duodenum just below the pancreatic duct and the first part of the jejunum. The bowel was then sectioned and the ends closed by inversion at the proximal end of the loop of duodenum thus formed. Recovery from this operation was completed by May 3, after which he was regularly fed and seemed to be quite normal.

May 12 to 16: All food withheld but water given freely.

May 16: Abdomen again opened, and the distal end of the duodenal loop was sectioned near the anastomosis and the two ends closed by inversion and suture. Thus there was formed a doubly occluded loop of the whole duodenum, while an entero-enterostomy reestablished the intestinal patency. Following this operation he was moderately sick for four or five days. He vomited somewhat. He was given all the water he would drink and no hypodermoclysis. At the end of a week he seemed entirely normal, ate greedily and had no trouble in digesting food. On the seventeenth day after the second operation, the abdomen was again opened. The duodenal loop was found tightly closed at both ends and practically empty. The mucosa was slightly congested, but otherwise quite normal.

Dog 108.—The same procedure was followed as in the previous case. Twenty-five days after the second operation the abdomen was opened and the loop examined. In this case there had been a reestablishment of the lumen from the distal end of the loop into the intestine. This had been possible because after sectioning the bowel, and inverting the two ends at the second operation, they had been fastened in apposition so as to prevent an intussusception. It is not conceivable that this could have resulted under one week, so that the dog lived during this time in good health with a doubly occluded duodenum.

In the whole series of doubly occluded duodena which we did, there was always a direct relation between the severity of the symptoms and the shortness of postoperative life on the one hand, and the amount of mucosal damage on the other.

The two cases cited above we submit as giving final proof to our contention that there is no fatal poison arising from the duodenum when there is no damage to the mucosa.

LOW OBSTRUCTION

Having demonstrated the causes of death in high intestinal obstruction we next turned our attention to a study of an obstruction lower down. In the following, the obstruction was produced by clamp in lower ileum or at cecum, and treated with small amounts of normal saline solution.

Dog 48.—Weight 13.5 kg. Fed twenty-four hours before operation. Clamp applied just above cecum.

December 12: Vomiting large amount of bile-stained fluid. Given water by mouth and 200 c.c. subcutaneously. Condition good.

December 13: Drank water greedily; 200 c.c. saline subcutaneously. Condition good. Temperature, 40. Pulse, 120. Vomiting somewhat.

December 14: Same.

December 15: Condition not so good. Acts sick. Pulse 145.

December 16: Very sick. Vomitus excessive. Bright red in color. Fecal in character and very foul. Pulse 180.

December 17: Died during night. Lived five and one-half days.

Autopsy.—Obstruction complete. Small abscess around clamp. No peritonitis. Very intense congestion throughout peritoneum. Kidneys and liver intensely congested.

Alimentary Tract: Stomach not distended, but mucosa somewhat congested. Duodenum the same. The entire ileum is greatly distended, wall thin, mucosa intensely congested. In lower part hemorrhagic areas are present, and in spots superficial areas of necrosis exist.

Damage to mucosa caused death.

Dog 50.—Weight 5 kg. Fed few hours before operation. Clamp applied just above cecum.

The postoperative condition exactly parallel to that of Dog 48. Remained in excellent condition for three days; then began to sicken and died after five and one-half days. He received from 150 to 210 c.c. water subcutaneously each day and drank in addition. Vomited every day.

Autopsy.—Obstruction complete. No peritonitis. Same changes found as in Dog 48. The ileum above the clamp was enormously distended and black in color. No actual necrosis seen. Kidneys and liver markedly congested.

Dog 52.—Fed twenty-four hours before operation. Weight 12 kg. Clamp applied just above cecum.

Postoperative behavior as in previous cases, except that he remained well for nine days, after which he grew sick. Received 200 c.c. of saline subcutaneously each day. Vomiting much less than in other cases.

Killed with ether on tenth day while in apparent fair condition.

Autopsy.—Obstruction complete. No pertitonitis. Upper bowel empty. Lower ileum much congested, markedly distended and containing a quantity of foul-smelling liquid, fecal matter. There was a marked peristalsis still present, and the fluid contents of the bowel could be seen continuously forced downward to the site of the obstruction. There was no marked evidence of the damage to the intestinal wall seen in the other cases. The liver showed some congestion, but otherwise the organs appeared normal.[24]

Cultures from the peritoneum, femoral blood, spleen and liver all proved negative, both anaerobically and aerobically.

This type of experiment was done six times, and the results accorded in every way with the three here described. Length of life exactly paralleled the lack of injury to the intestine as it had done in the high obstruction.

In the following low obstruction was treated with excess of saline subcutaneously:

Dog 85.—Clamp applied 62 cm. above the cecum. Normal saline, 400 c.c., was given subcutaneously at the end of the operation. This was repeated the next day, but in spite of it the dog only lived forty-six hours.

Autopsy.—No peritonitis. Obstruction complete. The bowel below the clamp is collapsed and empty. Above the clamp the bowel is dilated all the way to the stomach, being four or five times the normal diameter for a distance of 150 cm. This intestine shows very marked vascular changes throughout this length, as instanced by the presence of deep ecchymoses which involve all the coats except the peritoneal, through which, however, they are readily seen.

These changes are most marked on the convex border of the intestine, the surface at which the circulation would be naturally most disturbed by the excessive distention. There are no actual ulcers or necrotic areas seen in the mucosa.

Microscopical Examination.—The intestinal mucosa in the above areas is practically destroyed. There is edema throughout the entire wall of the bowel.

24. The length of life, and the absence of symptoms for such a long period, was due to lack of excessive vomiting and lack of damage to the intestine.

Between all the layers is a beginning purulent exudate with hemorrhages. These changes are probably of microbic origin.

Dog 86.—Clamp applied 40 cm. above the cecum. Dog remained perfectly well for three days. On the fourth day he began to act sick, and on the fifth day was greatly prostrated though still able to walk around the laboratory. During the five days the total output of urine and vomitus was 2,030 c.c., which was replaced by the administration of 2,050 c.c. of normal saline solution. The dog was killed with ether and the autopsy immediately done.

Autopsy.—No peritonitis. The clamp was tight and produced a complete obstruction. The bowel above the clamp was moderately distended for a distance of 50 cm., and shows no gross changes on peritoneal surface, except for congestion immediately above the clamp.

When the bowel is opened there are seen many scattered small areas where the mucosa is much damaged, in places showing a beginning necrosis. The content of the bowel is ordinary fluid fecal material.

Microscopical Examination.—There is an almost complete necrotic destruction of the mucosa at the points mentioned above. The entire thickness of the bowel wall is edematous and shows signs of acute exudative inflammation.

Kidneys: Acute parenchymatous inflammation is present.

Liver: Granular and lipoid degenerative changes are present.

Dog 88.—The clamp was applied 100 cm. above the cecum. This animal lived ten days, having remained in excellent condition for eight days. The vomiting was not so excessive as in the high obstruction, but was fecal in character and exceedingly offensive after the third or fourth day. The total output of urine and vomitus in ten days was 2,930 c.c., and the saline injections amounted to 3,900 c.c.

Autopsy.—There was no peritonitis. The obstruction was complete, the bowel below the clamp being collapsed and empty. Above the clamp the intestine was distended and filled with foul-smelling fluid feces. There was a slight purplish discoloration of the bowel for about 35 cm. above the obstruction. The mucosa appeared somewhat edematous, but there was no evidence of ulceration or necrosis.

Microscopical Examination.—The intestine shows congestion and edema. There are areas in which some necrosis is present, with exfoliation of the mucosal cells. No pyogenic infiltration is seen.

Liver shows only congestion.

Kidney shows congestion and some parenchymatous degeneration.

Dog 87.—The clamp was applied in the lower ileum. This animal lived in apparent perfect health for eighteen days, when he was killed. The total output of urine and vomitus was 3,950 c.c. and the injections of normal saline totaled 6,600 c.c.

It will be noted that the output of fluids is far less than in the high obstructions, and hence the quantities of saline injected may be reduced.

Autopsy.—There was no peritonitis. The obstruction was complete, the bowel below being empty and collapsed. The bowel above the clamp was moderately distended, but there was an absence of the discoloration, hemorrhages and other evidences of tissue damage seen in the other cases. On opening the intestine the mucosa appeared absolutely normal throughout, except for one small area 5 cm. in diameter, about 20 cm. above the clamp, where there was an ecchymosis.

Microscopical Examination.—The intestine shows only a slight congestion. There is an absence of the lesions found in the animals which died at an early date.

The kidneys and liver are entirely normal.

The study of low obstruction gives, therefore, results exactly like those found in the high obstruction. Dogs may be kept alive indefinitely by the injection of an excess of normal saline solution, if the intestinal

wall remains undamaged. Such injections, however, will not save life where there exist lesions in the mucosa which destroy the natural protection against the absorption of bacterial and other poisons from the lumen of the bowel.

It may be asked what actual evidence has been found that poisons enter the system even under these conditions. The evidence is indirect and inferential. It is plain that so long as the mucosa remains intact, and desiccation of the tissues is prevented, there is no absorption of poisons because the animals remain in perfect health. This does not mean that the stagnated intestinal contents would not prove poisonous if they could enter the blood. On the contrary, they are poisonous even after sterilization by boiling and filtering, as many investigators have shown. In every instance, however, in which serious symptoms of sickness develop and ultimately the animal dies, a damaged mucosa is found at autopsy. Study of this lesion shows it to be a destructive one. The mucosa is more or less destroyed and all the evidence is present that there is a bacterial inflammation in the intestinal wall. The severity of the symptoms varies directly and the length of life inversely with the extent of this damage. It is just as certain an inference that some poison is absorbed under these circumstances as any other of the many infective inflammatory lesions which we recognize as being the source of a toxemia, even though a bacteriemia is absent.

There are two points to be explained in comparing the high and low obstruction. First, why do the animals with the latter vomit a less quantity? Second, why cannot all the dogs with a low obstruction be kept alive by the administration of saline solution?

The vomitus is reduced in the low obstruction because there is a long surface of intestine through which absorption takes place. Similarly the dogs are less desiccated because the water content of the bile, gastric and intestinal juices reenter the body. On the other hand, there is practically no absorbing surface available for this purpose in the high obstructions, and the distance backward into the stomach being short, the latter organ rapidly fills and vomiting is imperative and almost incessant.

The explanation of the inability to save life in low obstruction is found in the mechanical conditions which exist. The small intestine above the obstruction becomes distended, and following this, kinking and tension on the mesentery develops. In this way there are areas in which severe circulatory disturbance is present, resulting in the very changes which we have found will cause death and which are uninfluenced by the administration of saline subcutaneously.

The results of our investigations of intestinal obstructions may be summarized as follows:

1. There are two factors at work in producing the symptoms and causing death in this condition.

First, the loss of water from the tissues, due to the excessive drain into the bowel in response to the irritation set up by the retained secretions. This water may be replaced by subcutaneous injection of normal saline solution, and the symptoms caused are thus entirely controlled, with a saving of life.

Second, the entrance into the circulation of poisonous materials, which occurs only when the mucosa of the bowel is damaged. This damage results largely from the trauma inflicted by the overdistention acting on the circulation, but possibly also by the chemical action of the digestive ferments stagnated above the obstruction. Such a damage having resulted, there occurs a bacterial invasion into the bowel wall with a death of tissue cells (which is invariably demonstrable by microscopical examination), and in this process the poisonous substances are elaborated. The action of these poisons is apparently not influenced by the administration of saline subcutaneously.

The absorption of a poison from any source whatsoever, so long as the mucosa remains intact, is not a factor.

2. The development of a bacteriemia as a cause of death is excluded.

CLINICAL APPLICATION

It is not always safe to transfer the findings of experimental work to clinical practice, and as yet we have had no extended opportunity to apply the teaching of our investigations to the bedside. There are certain applications, however, which seem justified. It has always been a matter of speculation why high intestinal obstruction in man is so much more acute than low obstruction. The explanation is found in the fact that water absorption is entirely stopped in the first, and in addition, enormous amounts of water are thrown out in the vomitus almost as soon as the obstruction occurs. A rapid desiccation takes place, and with it a severely damaged metabolism, as shown by the deranged urine output. These conditions obtain much more gradually when the obstruction is low in the bowel. Another factor is also found in the sudden enormous duodenal and gastric distention which mechanically damages the mucosa.

It is a familiar observation that a chronic obstruction may take place in the bowel and progress until the opening is only pin-point in size, yet no serious symptoms arise, even though there may be for many days cessation of fecal passage from the bowels. When, however, a complete closure supervenes, there arises immediate evidence of a severe condition. The well-known symptoms of acute intestinal obstruction are manifest. The explanation of this is found in the fact that so long

as gas can escape through the constriction, there is no distention of the intestine, and hence no damage to the intestinal wall, and no absorption of poisonous materials. The stoppage of gas, however, is immediately followed by both these conditions, and unless promptly relieved will result fatally. The practical application of the principles elaborated by the experimental work follows two lines. First, the water extracted from the tissues by the enormous outpouring into the bowel must be replaced. This can easily be done by means of a continuous hypodermoclysis administered under the skin in the pectoral region by use of needles of the ordinary hypodermic caliber, but longer. In this way the fluid is absorbed almost as rapidly as it enters, 1 or 2 liters being given without withdrawing the needles. The absorption will continue until the normal water content of the tissues is reestablished. Since, however, this has no influence on the symptoms resulting from a damaged intestinal wall, it is imperative to relieve the obstruction surgically as early as it is discovered. A second point of practical importance is derived from our experiments, that is, an answer to the question as to the advisability of opening the bowel, and either temporarily or permanently draining off the contents. Whenever there has resulted from the obstruction any considerable damage to the bowel wall above the constricted point, by reason of distention and circulatory changes, there is danger of serious symptoms arising from the absorption of materials resulting from these changes. Therefore, in the presence of such changes, the more rapid emptying of the bowel through an enterostomy than through the normal path, after relieving the obstruction, may be a wise thing to do. Whether the drainage of the intestine shall be made to continue after primarily emptying as much as may be possible of the distended bowel depends on the extent of the damage done. If this is great in amount, it may be advisable to establish an enterostomy, because there will be some farther accumulation of the poisons which will be absorbed unless there is a free exit for them. Such an enterostomy will be necessary if the contents of the bowel above the obstruction are blood-tinged, demonstrating a damage to the bowel wall. If it is simply fluid feces, the rapidly returning peristalsis, after the obstruction is released, will force the contents onward, and there will be no danger of poisonous absorption when once it gets into normal bowel below the obstruction, because the mucosa here possesses a defense against such absorption.

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27 East Sixty-Third Street—40 East Forty-First Street—55 East Sixty-First Street.

THE CLASSIFICATION OF AMEBAS

WITH OBSERVATIONS ON MORPHOLOGY AND LIFE CYCLE OF ENTAMOEBA COLI, CRAIGIA HOMINIS AND VAHLKAMPFIA LOBOSPINOSA *

CHARLES F. CRAIG, M.D.
FORT LEAVENWORTH, KAN.

Recent observations on the morphology of the amebas encountered in the human intestine, and on their life cycle, have shown conclusively that there are two species of entamebas parasitic in the intestine of man, *Entamoeba histolytica* and *Entamoeba coli,* and that in rare instances species belonging to the genus *Vahlkampfia,* normally free living, may lead a semiparasitic existence in the human intestine for a longer or shorter period of time. In view of these facts it has been necessary to revise our classification of amebas and in the following contribution I have adopted Calkins' classification and have recorded the latest observations on *E. coli, Craigia hominis* and *Vahlkampfia lobospinosa.* If some of the statements contained in the paper appear somewhat dogmatic I can only plead in excuse the fact that they are personal opinions based on years of careful study of the organisms in question.

CLASSIFICATION AND NOMENCLATURE

It would be of little interest to describe the efforts made at the classification of the amebas occurring in man prior to the researches of Schaudinn. In 1903[1] he divided these organisms into two species, *E. histolytica* and *E. coli.* At present several classifications are advocated by different authorities and it is difficult to select that which is accepted by the greatest number of protozoologists. The work of Musgrave and Clegg[2] on the cultivation of amebas from man early made it evident that amebas occur in the intestine and in the feces, as well as in the pus of liver abscesses, that were not identical with either *E. histolytica* or *E. coli,* and their conclusion that these cultivated amebas could cause dysentery resulted in the greatest confusion in classification and nomen-

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* From the Department Laboratory, Central Department, U. S. Army.

1. Schaudinn, F.: Untersuchungen ueber die Fortpflanzung einiger Rhizopoden, Arb. a. d. k. Gsndhtsamte, 1903, xix, 547.

2. Musgrave, W. E., and Clegg, M. T.: Amebas: Their Cultivation and Etiologic Significance, Bull. Govt. Lab., Biolog. Lab., Manila, 1904, 18.

clature. The work of Hartmann[3] and Whitmore,[4] as well as that of many other investigators, in proving that these cultivated amebas are not entamebas at all and have nothing to do with the production of dysentery, has resulted in a clearer conception of the entire question of classification of the parasitic amebas, or entamebas, and their relation to infection and the production of disease.

At present it is admitted that the old genus *Amoeba* should be split into several genera, some of these living free in nature, while others are either partly or wholly parasitic in their life habits. It is also recognized that some of the free-living forms frequently contaminate the food of man and thus reach the intestinal canal, where they may be demonstrated in the feces, generally in the encysted stage of development, but sometimes as vegetative forms, and it is believed that certain of these free-living species may actually become temporary parasites of man or other animals. The amebas that have been cultivated from the feces belong to these free-living species, and have either reached the feces through being swallowed in infected food or water, or by aerial contamination. These species differ entirely from the entamebas both in morphology and life history. Although under the action of certain stimulins in artificial culture-mediums these organisms may show developmental stages similar to those observed in entamebas, and their morphology may become markedly modified, as proved by Calkins and Williams,[5] a careful study of these modified organisms will show that, after all, the nuclear structure is quite different from that of either *E. histolytica* or *E. coli*, and that there is no reason to believe that the latter species ever are transformed into the free-living species. That the genus *Vahlkampfia* contains species that can be cultivated from the intestine or feces of man is undoubted, and that under cultivation these species may undergo marked modifications in morphology and life history is true, but that they are identical with true entamebas is disproved by the observations of nearly all authorities who have worked with both genera.

The modern classification of amebas has been attempted by many protozoologists, and Chatton,[6] Alexeieff,[7] Doflein,[8] and Hartmann[3] have each proposed classifications that possess advantages. In this paper,

3. Hartmann, M.: Article "Die Dysenterie Amoeben" in Von Prowazek's Handbuch der Pathogenen Protozoen, 1912, i, 51.

4. Whitmore, E. R.: Parasitaire und Freilebende Amoeben aus Manila und Saigon, etc., Arch. f. Protistenk., 1911, xxiii, 71.

5. Williams, Anna, and Calkins, G. N.: Cultural Amoebae, a Study in Variation, Jour. Med. Research, 1913, xxix, 43.

6. Chatton, E., and Lalung-Bonnaire, P.: Amibe limax (Vahlkampfia n. gen.) dans l'intestin humaine, Bull. Soc. path. exot., 1912, v, 13.

7. Alexeieff, A.: Sur les caractères cytoligiques et la systématique des amibes du groupe Limax, Bull. Soc. zool. de France, 1912, v, 55.

8. Doflein, F.: Lehrbuch der Protozoenkunde, edit. 3, 1911, p. 560.

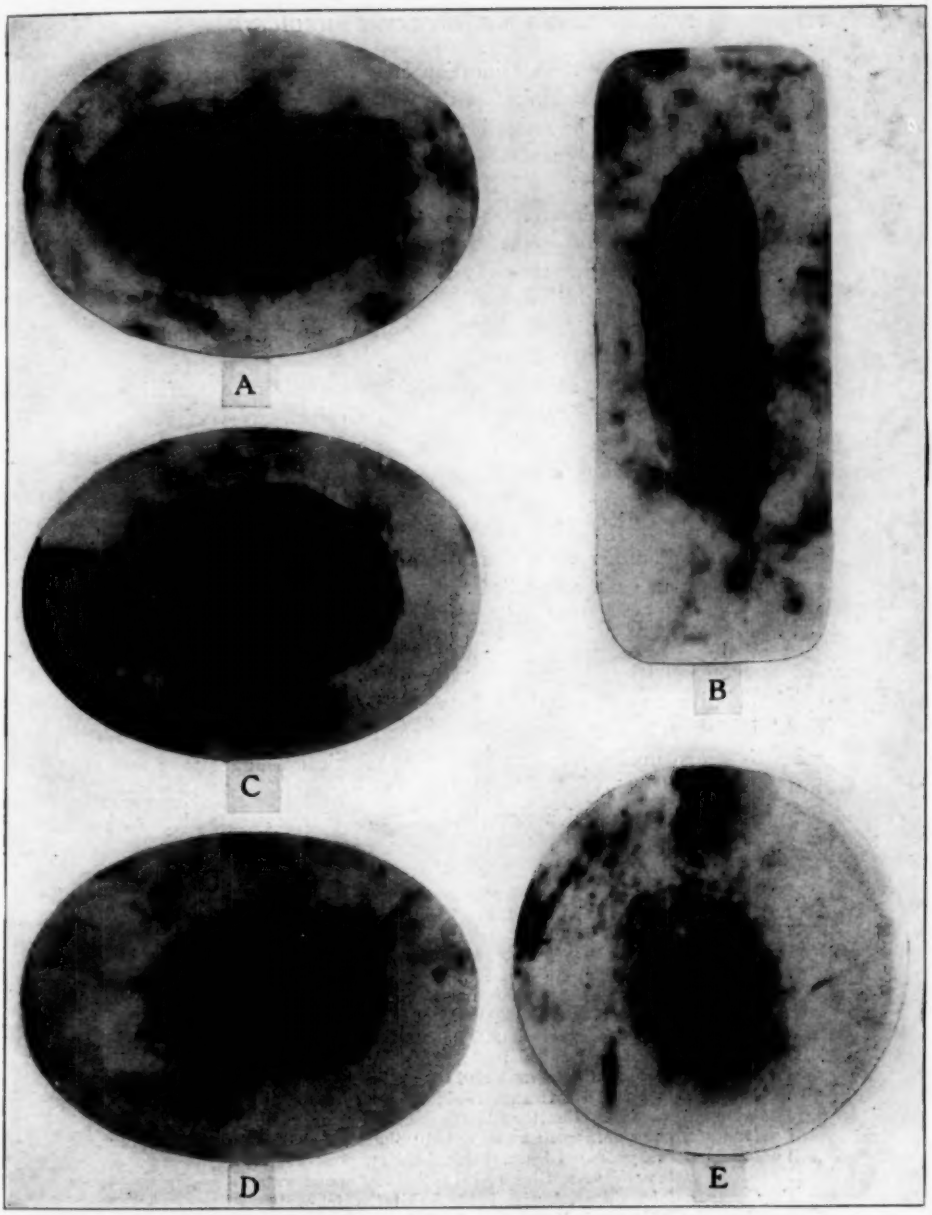

Fig. 1.—Vegetative forms of *Entamoeba coli;* from photomicrographs × 1,200; all specimens stained by Mallory's ferric-chlorid-hematoxylin method. *A*, large-sized organism with characteristic nucleus; *B*, type of nucleus before division begins; *C*, typical nucleus; *D*, nucleus with chromatin on inner border of nuclear membrane; *E*, typical karyosome, granular appearance of the nuclear membrane, linin net-work and intranuclear chromatin.

however, I shall follow the clasification adopted by Calkins,[9] because I believe it to be the most logical at the present stage of our knowledge of the amebas. It must be remembered, however, that any classification, at present, must be purely tentative until the life cycle of every organism contained in it is known, and this is true of only a very few of the so-called species of amebas. It should, therefore, be clearly understood, that while I adopt for the present the classification of Calkins, and while I shall list all described species of amebas under their respective genera in this classification, I believe that in time it will have to be changed, and that many of the described species will eventually be found to be identical with those better known.

Calkins divides the old genus *Amoeba* into seven genera, as follows: *Amoeba, Vahlkampfia, Nagleria, Paramoeba, Craigia, Trimastigamoeba,* and *Entamoeba.* Of these seven genera the only ones of interest to us in our discussion of the parasitic amebas of man are: *Vahlkampfia, Craigia, Trimastigamoeba* and *Entamoeba.*

Genus *Vahlkampfia,* Chatton, 1912: Chatton established this genus in 1912, as containing certain small amebas originally classed in the genus *Amoeba* under the so-called limax type, and excluding organisms having a flagellate stage of existence.

Amebas of this genus are characterized by the presence in each of one or more nuclei and one or more contractile vacuoles. They divide by simple division or a primitive form of mitosis, forming a uninucleate cyst. Under certain abnormal cultural conditions the cyst may contain more than one nucleus, and marked morphological changes may occur in the nucleus (Craig). The nucleus in the vegetative stage contains a very large karyosome which sometimes shows a distinct centriole. Habitat: water, earth, and occasionally in man or other animals.

The following species are included in this genus:

Vahlkampfia limax, Dujardin, 1841; habitat, fresh water.
Vahlkampfia guttula, Dujardin, 1841; habitat, pond-water.
Vahlkampfia polypodia, Schultz, 1875; habitat, pond-water.
Vahlkampfia binucleata, Gruber, 1884; habitat, pond-water.
Vahlkampfia diploidea, Hartmann and Nagler, 1908; habitat, garden earth.
Vahlkampfia albida, Nagler, 1909; habitat, garden earth.
Vahlkampfia horticola, Nagler, 1909; habitat, garden earth.
Vahlkampfia lacustris, Nagler, 1909; habitat, fresh water.
Vahlkampfia froschi, Hartmann, 1907; habitat, fresh water.
Vahlkampfia lacertae, Hartmann, 1907; habitat, in intestine of lizards.
Vahlkampfia mucicola, Chatton, 1909; habitat, ectoparasitic on fish.
Vahlkampfia paedophthora, Caullery, 1910; habitat, eggs and embryos, crabs.
Vahlkampfia tachypodia, Glaser, 1912; habitat, pond-water.
Vahlkampfia lamellipodia, Glaser, 1912; habitat, pond-water.
Vahlkampfia lobospinosa, Craig, 1912; habitat, ditch-water.
Vahlkampfia whitmorei, Hartmann, 1913, habitat, swamp-water.

9. Calkins, G. N.: Genera and Species of Amoeba, Tr. Fifteenth Internat. Cong. Hyg. and Demog., 1912, ii, 287.

While most of the species so far described in this genus are free living, there is excellent evidence on record that some of them may become parasitic under certain conditions. Of those mentioned in the list, the one described by Hartmann as *Vahlkampfia lacertae* is certainly a parasitic species, as are *V. mucicola* and *V. paedophthora*. Several observers have already noted the occurrence of typical *Vahlkampfiae* in the feces of man in both the vegetative and cystic stages of development, when the question of outside contamination could be eliminated. I have seen some beautiful preparations containing a typical *Vahlkampfia* obtained by Dr. W. M. James from the feces of a patient observed at Panama. The organisms are in both the vegetative and cystic stages of development and were observed over a considerable period of time, so that the question of contamination of the feces was ruled out. It is interesting to observe that these amebas were easily distinguishable from either *E. histolytica* or *E. coli* in both the vegetative and encysted stages of development.

It is probable that nearly all species of *Vahlkampfia* may be cultivated on artificial mediums, and it is this fact that has given rise to so much confusion regarding the cultivation of the true parasitic amebas. These organisms frequently contaminate fecal or other material derived from patients suffering from entamebic dysentery, and may thus be obtained in cultures. Amebas belonging to this genus have been cultivated by many observers, notably Musgrave and Clegg,[2] in impure culture, and by Williams[10] in pure culture, but the claims made by these investigators that the amebas so cultivated are identical with the true entamebas are unsupported by any adequate evidence. It is now admitted by all competent observers that neither *E. histolytica* nor *E. coli* has been cultivated.

The *Vahlkampfia* named in the above list as *Vahlkampfia lobospinosa* is a true free-living ameba that was first cultivated by Musgrave and Clegg from the feces of a dysentery patient. It has since been obtained in pure culture by Williams. This organism, as shown by Calkins and Williams,[5] when growing in pure culture, undergoes marked morphological and cyclical changes in response to certain cultural conditions, but in the human feces it possesses the morphology of a true *Vahlkampfia*, and even in the cultures is not indistinguishable from the entamebas found in man, as is well shown in the photomicrographs accompanying the paper by these observers. This species has been under cultivation for several years, and the cysts have been found to resist drying for over one year, a fact that alone proves it to be entirely distinct from the entamebas of the intestine of man, for all the evidence of recent researches

10. Williams, Anna: Pure Cultures of Amoebae Found in Intestines of Animals, Studies Research Lab., New York Board of Health, 1912, vi, 298.

proves that the cysts of these entamebas have comparatively little resistance.

While, as has been stated, species belonging to this genus have been observed apparently parasitic in man, and while, in some cases, they may be cultivated from the feces, the pus of liver abscesses, and other sources, there is no evidence that demonstrates that they are pathogenic or that they can live for any considerable time within the human intestine. In the vast majority of instances they reach the material from which they are cultivated from the air, as proved by Liston[11] and Wells,[12] or by passing through the intestinal canal in contaminated food or water, generally in the encysted condition. It is true that, in rare instances, species of *Vahlkampfia* may become parasitic for a short time in the human intestine, but this is really an abnormal mode of existence, no symptoms of disease are produced, and they may be easily distinguished from the true entamebas by the large nuclear karyosome, the uninucleate cyst, and the presence of at least one contractile vacuole. Their presence in the intestine is chiefly of interest from the point of view of evolution, as indicating the possible origin of the true parasitic amebas.

Genus *Craigia*, Calkins, 1912: In 1912 Calkins[9] established the genus *Craigia* to include the ameba described by myself under the name *Paramoeba hominis*, in 1906. This ameba was originally placed in the genus *Paramoeba* because of its resemblance to Schaudinn's *Paramoeba eilhardi*, but Calkins has shown that it should be placed in a new genus, an opinion previously expressed by Doflein.[8]

The type-species of this genus is *Craigia hominis* (*Paramoeba hominis*) a parasitic ameba that I have observed in twelve patients suffering from a chronic form of diarrhea. It is characterized by a nucleus, an accessory nuclear body, and by an ameboid and flagellate stage of development. Multiplication occurs in the ameboid stage by simple division and by the formation of cysts containing numerous minute flagellated organisms. In the flagellate stage of development multiplication occurs by longitudinal division, preceded by division of the accessory body and the nucleus. Habitat, the intestine of man.

The following species are included in this genus:

Craigia hominis, Craig, 1906; emend. Calkins, 1912.
Craigia pigmentifera, Grassi, 1881; habitat, body cavity of *Chaetognathi*.
Craigia chaetognathi, Grassi, 1881; habitat, body cavity of *Chaetognathi*.

It is interesting to note that all of the species so far described for this genus are parasitic, *Craigia hominis* being parasitic in man, while

11. Liston, W. Glen: A Note on Early Stages of Nuclear Division, Quart. Jour. Micr. Sc., 1911, lvii, 279.

12. Wells, R. T.: Aerial Contaminations as a Fallacy in the Study of Amoebic Infections by Cultural Methods, Parasitology, 1911, iv, 204.

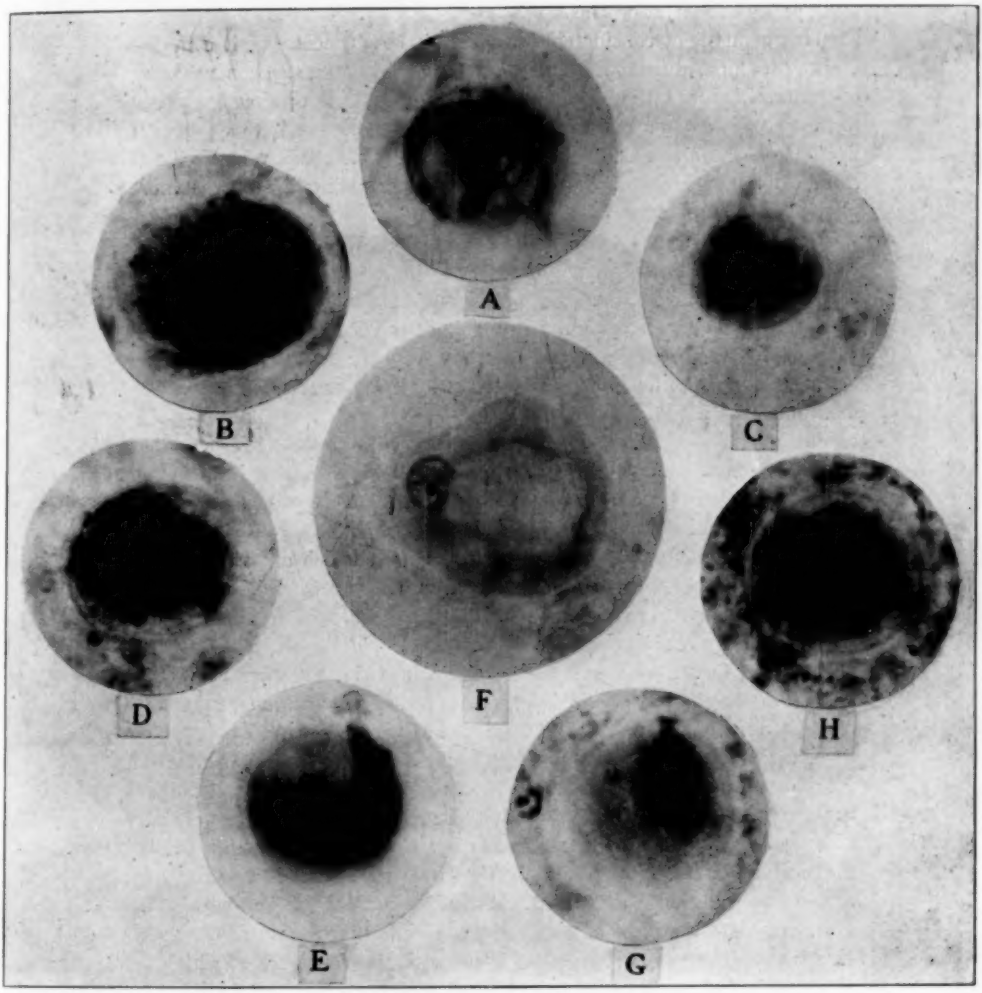

Fig. 2.—Precystic and cystic form of *Entameba coli;* from photomicrographs × 1,200; *A*, precystic form in which the nucleus is more than usually typical; *B*, division of the karyosome into two portions; *C*, precystic form showing change in type of nucleus and slight elongation of karyosome; *D*, precystic form containing yeast cells; *E*, typical cyst showing nucleus in which the karyosome has apparently divided; *F*, very large cyst showing large vacuole and nucleus in which the karyosome has elongated preparatory to division; *G*, cyst containing six nuclei; eight were present but two are out of focus; *H*, cyst containing three definite nuclei. Four were present, but one does not show as it lies out of focus.

Craigia pigmentifera and *Craigia chaetognathi* are both parasitic in certain species of marine worms.

This genus is of considerable interest to the physician, as it contains a species which, during the ameboid stage, may be easily confused, by one unfamiliar with the protozoa of the intestine, with amebas belonging to the genus *Vahlkampfia* or *Entamoeba*, and in the flagellate stage with *Trichomonas hominis* or other intestinal flagellates. This mistake, however, could never be made by any one well acquainted with the morphology of the parasites mentioned.

Genus *Trimastigamoeba*, Whitmore, 1911: Whitmore[13] established this genus, in 1911, to include an ameba isolated by himself in cultures from tap-water, in Manila. The type-species is *Trimastigamoeba philippinensis*, and is characterized by a nucleus of the limax type, a contractile vacuole, and the occurrence, under certain conditions, of flagellate forms having three equal flagella. The species is easily cultivated on suitable mediums, and is the only one so far placed in the genus. This ameba is of interest to physicians, because it may contaminate fecal or other material from human sources and appear in cultures prepared from such material. There is no evidence that this species occurs as a parasite in man or other animals.

Genus *Entamoeba*, Leidy, 1879: This genus, which contains most of the parasitic amebas of man and other animals, was established in 1879 by Joseph Leidy,[14] who used the spelling *Endamoeba* in the description of the genus. The type-species is *Entamoeba blattae*, a parasite of the cockroach. Casagrandi and Barbagallo,[15] in 1897, unaware of Leidy's genus *Endamoeba*, established for the parasitic amebas the genus *Entamoeba*, and this is the spelling employed at present.

The generic characters are as follows: Size comparatively small, from 5 to 80 microns. Ectoplasm and endoplasm distinct when the organisms are moving and often when motionless; contractile vacuole generally absent; nucleus with definite karyosome and centriole; nuclear division mesomitotic and mitotic; reproduction by simple division, schizogony, and cyst formation with the production of several amebas within the cyst. Habitat, the body of man or other animals, generally in the digestive tract. The genus contains only parasitic species, some of which are pathogenic, but most live merely as commensals in the intestine of their host. None of the species have been artificially cultivated.

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13. Whitmore, E. R.: Studien über Kulturamoeben aus Manila, Arch. f. Protistenk., 1911, xxiii, 81.

14. Leidy, Joseph: Fresh-Water Rhizopods of North America, Rep. U. S. Geol. Surv., Washington, 1879, xii, 1.

15. Casagrandi, O. G., and Barbagallo, P.: Richerche biologiche e cliniche sull' Amoeba colix, Boll. d. Accad. Gioenia di sc. nat. in Catalonia, n. s., 1895, xxxix, 4.

A very large number of species of amebas have been placed in this genus, and new species are being discovered almost monthly, but, as I have already stated, further study will prove many of these to be identical with species already studied. I have placed in the list that follows, however, all the species reports of which I have been able to collect in the literature, but I have indicated those identified with other species. The list is arranged alphabetically, and the name of the host follows that of the parasite.

Entamoeba africana, Hartmann, 1907; identical with *Entamoeba histolytica;* host, man.
Entamoeba blattae, Leidy, 1879; host, cockroach.
Entamoeba aulastomi, Noller, 1912; host, eagle.
Entamoeba bovis, Liebetanz, 1905; host, cattle.
Entamoeba brasiliensis, Baurepaire Aragao, 1912; host, man.
Entamoeba buccalis, von Prowazek, 1907; host, man.
Entamoeba butschlii, von Prowazek, 1912; host, man.
Entamoeba cobayae, Walker, 1908; host, guinea-pig.
Entamoeba coli, Loesch, emend. Schaudinn, 1903; host, man.
Entamoeba dentalis, Braun, 1883; identical with *E. buccalis;* host, man.
Entamoeba duboscqi, Mathias, 1913; emend. Craig, 1913; host, monkey.
Entamoeba dysenteriae, Councilman and Lafleur, emend. Craig, 1905; identical with *E. histolytica;* host, man.
Entamoeba gingivalis, Braun, 1895; identical with E. buccalis; host, man.
Entamoeba hartmanni, von Prowazek, 1912; host, man.
Entamoeba histolytica, Schaudinn, 1903; host, man.
Entamoeba intestinalis, Walker, 1908; host, cat.
Entamoeba kartulisi, Doflein, 1901; host, man.
Entamoeba lacertae, Hartmann, 1907; identical with *Vahlkampfia lacertae;* host, lizard.
Entamoeba lagopidis, Fantham, 1910; host, moor hen.
Entamoeba legeri, Mathias, 1913, emend. Craig, 1914; host, monkey.
Entamoeba maxillaris, Kartulis, 1906; identical with *E. kartulisi;* host, man.
Entamoeba minuta, Elmassian, 1909; identical with *E. histolytica*; host, man.
Entamoeba miurai, Ijima, 1898; host, man.
Entamoeba mortinatalium, Smith and Weidman, 1910; host, man.
Entamoeba muris, Grassi, 1881; host, mouse.
Entamoeba nipponica, Koidzumi, 1909; identical with *E. coli;* host, man.
Entamoeba nuttalli, Castellani, 1908; host, monkey.
Entamoeba phagocytoides, Gauducheau, 1907; host, man.
Entamoeba pithreci, von Prowazek, 1912; host, monkey.
Entamoeba polecki, von Prowazek, 1912; host, swine and man.
Entamoeba pulmonalis, Artault, 1898; host, man.
Entamoeba ranarum, Grassi, 1879; host, frog.
Entamoeba salpae, Alexeieff, 1912; host, fish.
Entamoeba schaudinni, Lesage, 1908; host, man.
Entamoeba suis, Hartmann; host; swine.
Entamoeba testudinis, Hartmann, 1910; host, turtle.
Entamoeba tetragena, Viereck, 1907; identical with *E. histolytica*; host, man.
Entamoeba tropicalis, Lesage, 1908; identical with *E. coli*; host, man.
Entamoeba undulans, Castellani, 1904; host, man.
Entamoeba urogenitalis, Baelz, 1883; host, man.
Entamoeba williamsi, von Prowazek, 1911; host, man.

It is more than probable that at least two-thirds of these species here given as parasitic in man are not valid species, but, as I have not had the opportunity of studying them, I include in the list all of the species

of entamebas that have been described. Of these, however, there are really only two species that are of great interest to the physician; that is, *E. histolytica* and *E. coli;* the former is the common pathogenic species causing entamebic dysentery and liver abscess in man, while the latter is a harmless commensal, found in a varying, but usually large proportion of healthy, as well as diseased individuals, in almost every part of the world.

A word of explanation is necessary in regard to the classification of the so-called *E. tetragena.* This entameba was first described by Viereck,[16] in 1907, as a distinct species causing dysentery in man, and his observations were quickly confirmed by Hartmann[2] and Prowazek, Whitmore,[17] and myself;[18] but recent researches have proved conclusively that this species is really identical with *E. histolytica,* a portion of the life cycle of the latter species having been erroneously described by Schaudinn and his followers, and first correctly described by Viereck in his description of *E. tetragena.* It therefore follows that *tetragena* ceases to exist as a distinct species and the name must be eliminated from our nomenclature.

It has also been shown that *E. minuta* is identical with *histolytica,* and that *E. nipponica* and *E. tropicalis* are identical with *E. coli,* so that these three names also should be eliminated as specific names of entamebas.

Since the discovery that *E. tetragena* was identical with *E. histolytica,* the credit for which belongs to Walker,[19] nearly all students of the subject who have had sufficient material on which to work are agreed that two well-defined species of entamebas occur in the intestine of man. Since 1905 I have consistently maintained the specificity of these two entamebas; that is, *E. histolytica* and *E. coli,* and in my first paper,[20] and since, have insisted that it is possible to differentiate them in practically every case of infection. Many observers have confirmed my conclusions, but the recent work of Walker and Sellards[21] has conclusively proved my statements regarding the pathogenic nature of *histolytica* and the non-pathogenicity of *coli,* and also my conclusions regarding the lack of identity of cultural amebas and those parasitic in man. By experi-

16. Viereck, H.: Studien über die in den Tropen erworbene Dysenterie, Arch. f. Schiffs u. Tropen-Hyg., 1907, ii, 1.

17. Whitmore, E. R.: Parasitaire und freilebende Amoeben aus Manila und Saigon, Arch. f. Protistenk., 1911, xxiii, 71.

18. Craig, Charles F.: Entamoeba Tetragena as a Cause of Dysentery in the Philippine Islands, THE ARCHIVES INT. MED., 1911, vii, 362.

19. Walker, E. L.: A Comparative Study of the Amoebae of the Manila Water-Supply, etc., Philippine Jour. Sc., Med. Sec., 1911, vi, 259.

20. Craig, Charles F.: Observations Upon Amebas Infecting the Human Intestine, with a Description of Two Species, Entamoeba Coli and Entamoeba Dysenteriae, Am. Med., Philadelphia, 1905, ix, 854, 897 and 936.

21. Walker, E. L., and Sellards, A. W.: Experimental Entamoebic Dysentery, Philippine Jour. of Sc., Sec. B, Trop. Med., 1913, viii, 253.

ments on man these authors have proved that the amebas that can be cultivated from water, from the feces of man and other animals, and from other sources, are incapable, when ingested, of producing lesions in man, and that they are not entamebas but free-living amebas which do not produce dysentery, while, as already stated, their experiments prove

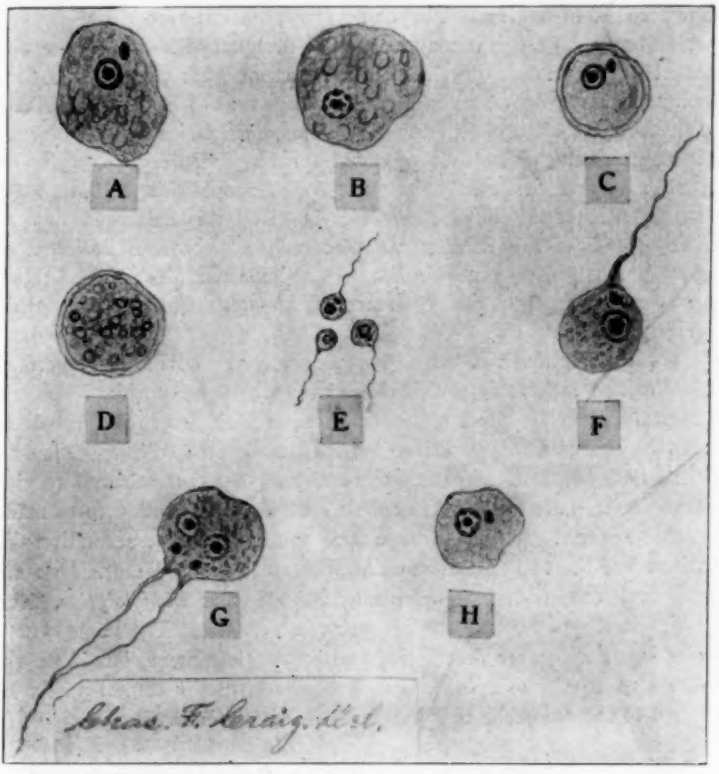

Fig. 3.—*Craigia hominis*, from pencil drawings, × 1,200. *A*, typical organism in amebic stage (note structure of nucleus and accessory nuclear body); *B*, absence of accessory nuclear body and different type of nucleus; *C*, cyst showing one nucleus; *D*, cyst showing granular appearance of cytoplasm and the presence of numerous nuclei; *E*, young flagellate form; F, fully developed flagellate form. (Note absence of undulating membane, the single flagellum, the nucleus, and accessory nuclear body); *G*, dividing flagellated form the flagellum being partly divided, while the nucleus and accessory nuclear body have divided; *H*, flagellate form after loss of flagellum and just before becoming ameboid.

that *E. histolytica* produces dysentery in man, and *E. coli* is a harmless commensal in the human intestine. While all of the essential points covered in Walker and Sellard's work have been previously proved by

experiments on the lower animals, their results possess the very great advantage of having been worked out on man, the natural host of *histolytica* and *coli*, and, in my opinion, settle for all time the question of the relation of parasitic and cultural amebas to disease in man.

At present a considerable amount of controversy has arisen regarding the proper name for the common dysentery-producing entameba, usually known as *E. histolytica*. The name *tetragena* must be dropped as a specific name, although it may be used to designate the type of nucleus present during certain developmental stages of *histolytica*. This conclusion is admitted by nearly all protozoologists; but Doflein and Hartmann favor substituting the specific name *dysenteriae* for *histolytica*, this name having been first proposed for the dysentery entameba by Councilman and Lafleur. This is not a new proposition, for in 1905[20] I suggested that the name *dysenteriae* be given this entameba, but, as Stiles later showed that this name was merely a synonym of *coli*, and as Schaudinn had already used *coli* as the specific name for the harmless entameba of man, I adopted his name, *E. histolytica*, for the pathogenic species.

While it is undoubtedly true that Councilman and Lafleur described *E. histolytica* in part in their exhaustive work on entamebic dysentery, it is perfectly evident to any one who studies their description carefully that they confused this parasite with the harmless *E. coli*, and failed to differentiate between the pathogenic and harmless entamebas or to give morphological data sufficient to enable such a differentiation to be made by other workers. In view of these facts and the fact that Schaudinn *did* differentiate the two species mentioned, I am convinced that, if we are to be consistent in our nomenclatural rules, the name *E. histolytica*, given by him to the pathogenic species, must be retained. The name *dysenteriae* is a much more descriptive and appropriate name, but if we are to be true to the accepted rules of nomenclature it cannot justly be applied to the entameba now known as *E. histolytica*.

OBSERVATION ON E. COLI, LOESCH, 1875; EMEND. SCHAUDINN, 1903

It was long known that amebas were frequently present in the feces of healthy persons and in those of patients suffering from other diseases than dysentery, Grassi[22] being one of the first to describe these amebas. His work was followed by that of Calandruccio,[23] Schuberg,[24] Strong and

22. Grassi, G. B.: Dei Protozoi parasiti e specialmente di quelli che sons nell' uomo, Gaz. Med. ital., Lombardie, 1879, xxxix, 445.

23. Calandruccio, S.: Animali parasiti dell' uomo in Sicilia, Atti d. Accad. Gioenia di sc. nat. in Catania, 1889, lxvi, 95.

24. Schuberg, A.: Die parasitischen Amoeben des menschlichen Darmes, Centralbl. f. Bakteriol., 1893, xiii, 598, 654 and 701.

Musgrave,[25] and Casagrandi and Barbagallo.[15] In 1903, Schaudinn[1] accurately described this entameba, and his observations were confirmed by myself in 1905.[26] Important papers dealing with this parasite have since appeared by Werner[27] and Hartmann and Whitmore.[28]

Several observers since Schaudinn have described as new species entamebas occurring in healthy, as well as diseased individuals, that answer closely to the description of *E. coli*. Among these may be mentioned *E. tropicalis*, described by Lesage[29] in 1908; *E. nipponica*, described by Koidzumi[30] in 1909, and *E. williamsi*, described by von Prowazek[31] in 1911. Recent observations have shown that all these so-called species are identical with *E. coli*, and it is probable that some other species of entamebas that have been described in the intestine of man are also identical with this parasite.

The recognition of this species is of great importance because of its world-wide distribution, the fact that it is a harmless parasite, and the possibility of confusing it with the pathogenic *E. histolytica*. Our statistics of the actual occurrence of entamebic dysentery in all parts of the world are greatly vitiated by the non-recognition of this harmless species, many authorities making a diagnosis of entamebic dysentery whenever an ameba was found in the stools, without regard to the species present. It is now evident that a diagnosis of entamebic dysentery based on anything less than the recognition of *E. histolytica* in the feces cannot be considered as authentic, and if this test had been applied to the diagnosis of the disease in the past it is probable that our conceptions of its frequency in certain localities would be greatly modified.

E. coli has a very wide geographical distribution, being most frequently observed in tropical and subtropical regions, but being by no means infrequent in the temperate zones. In discussing the relation of this organism to disease the distribution will be more fully considered, but it may be stated here that it has been found wherever determined efforts have been made to demonstrate it, and this fact makes its recognition of prime importance in the discussion of entamebic infection.

25. Strong, R. T., and Musgrave, W. E.: Preliminary Note Regarding the Etiology of the Dysenteries of Manila, Rep. Surg. Gen. U. S. Army, 1900, p. 251.

26. Craig, Charles F.: Observations Upon Amoebae Infecting the Human Intestine, Etc., Am. Med., Philadelphia, 1905, ix, 854, 897 and 936.

27. Werner, H.: Entamoeba Coli, Handbuch der path. Protozoen, von Prowazek, i, 67.

28. Hartmann, M., and Whitmore, E. R.: Untersuchungen über Parasitische Amoeben: Entamoeba coli, Arch. f. Protistenk., 1912, xxiv, 182.

29. Lesage, A.: Culture de l'amibe de la dysenterie des pays chauds, Ann. de l'Inst. Pasteur, 1909, xviii, 9; Bull. Soc. de path. Exot., 1908, v, 104.

30. Koidzumi, M.: On a New Parasitic Amoeba, Entamoeba Nipponica, found in the Intestine of Japanese, Centralbl. f. Bakteriol., Orig, 1909, li, 650.

31. Von Prowazek, S.: Beitrage zur Entamoeba Frage (Entamoeba williamsi), Arch. f. Protistenk, 1911, xxii, 345.

Morphology.—Like *E. histolytica,* this species varies greatly in morphology at different stages in its life cycle, and this cycle may also be divided into the vegetative stage, the precystic stage and the cystic stage, in each of which a description of the special morphology is necessary.

Morphology in the Vegetative Stage.—In this stage of development *E. coli* varies greatly in size, very large and very small organisms being frequently observed in the same preparation. The living vegetative parasites vary from 15 to 50 microns in diameter, and rarely may be larger, but the average diameter is about 25 to 35 microns. The cytoplasm is divided into two portions, the ectoplasm and endoplasm, but the distinction is not possible save when the organisms are moving. In this species the ectoplasm does not have the glass-like appearance of the ectoplasm of *E. histolytica,* but approaches much more closely to the endoplasm in appearance, so that even when moving the ectoplasm is not nearly as distinct in *coli* as it is in *histolytica,* a very valuable differential point. The *cytoplasm* contains a visible nucleus, appearing as a spherical body bounded by a refractile granular membrane and containing granules or small masses of refractile chromatin, generally arranged as a well-marked karyosome. The nucleus of this species, even in the living condition, appears larger than does the *tetragena* type of nucleus of *E. histolytica,* and this is more easily demonstrated in the living parasite. The endoplasm appears very granular and contains numerous vacuoles, bacteria, and crystals taken up from the feces. In contradistinction to *E. histolytica,* this parasite is not phagocytic for red blood-corpuscles, even when the feces contain much blood, nor have I been able to demonstrate this phenomenon by adding fresh blood to feces containing *E. coli,* although phagocytosis of the blood-corpuscles may often be observed if blood be added to fecal material containing *E. histolytica.* I have observed very rarely an entameba of this species with one or two red blood-corpuscles within the endoplasm, but I have never observed them filled with erythrocytes as in the case of *histolytica,* and I believe that when this process does occur in *coli* it is purely accidental, while in *histolytica* it is a normal process in the nutrition of the organism. At any rate, the point is of great value in the differentiation of the two species, for one is quite safe in regarding any entameba containing red blood-corpuscles as a pathogenic parasite, presumably *E. histolytica.*

The motility of *E. coli,* in the vegetative stage, is much less marked than that of *E. histolytica,* although it may be quite active in the larger organisms in freshly voided feces. To one who has had experience in the study of living specimens of both species, the difference in motility is very marked, *E. coli,* even when most active, being sluggish in comparison with the average vegetative specimen of *E. histolytica.* The movement is also less progressive in character and more apt to be indefinite in direction.

In stained preparations, (wet fixation and iron-haematoxylin) during the vegetative stage of development, the cytoplasm stains a brownish or grayish color and appears granular in consistence, containing bacteria, crystals, and generally a considerable number of vacuoles, but the most striking object is the nucleus, which in this species is much larger than either the histolytica or tetragena type of nucleus of *E. histolytica*, in the vast majority of organisms examined, and much richer in chromatin. The nucleus is spherical and situated

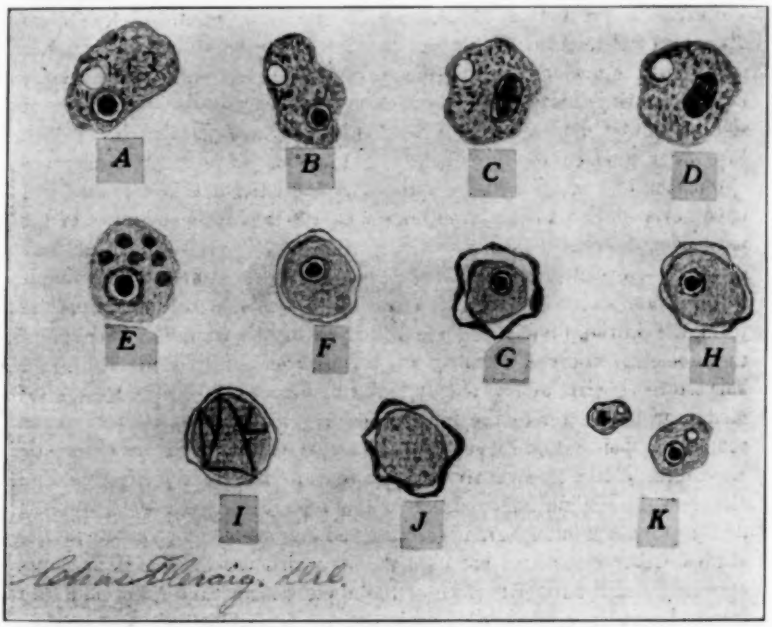

Fig. 4.—Vegetative forms of *Vahlkampfia lobospinosa*, from pencil drawings, × 1,200; *A*, shows large contractile vacuole near the nucleus, and typical nucleus (note the large, deeply staining karyosome); *B*, the contractile vacuole near the periphery of the cytoplasm; *C*, division of the nucleus; note the polar bodies and equatorial plate; *D*, nuclear division; *E*, organism containing yeast-cells; note the granular appearance of the nuclear membrane; *F*, typical young cyst; *G*, old cyst; note thick cyst-wall which is folded in places, a very characteristic appearance of the old cysts; *H*, *I*, *J*, cysts; *I* and *J*, degenerated, the nucleus having disappeared; *K*, young organisms shortly after liberation from cyst.

within the endoplasm. The nuclear membrane is thick and stains intensely black with hematoxylin. It is even thicker than the membrane of the tetragena type of nucleus in *E. histolytica* and presents on its inner surface granules, or more frequently, clumps of chromatin. The

karyosome is situated at or near the center of the nucleus and appears most frequently as a spherical mass of chromatin, stained almost black, and containing no well-defined centriole. In some instances a centriole may be distinguished as a more deeply staining dot of chromatin near the center of the karyosome. In this species a certain series of cyclical changes occur in the nucleus, but they are not so pronounced as in the nucleus of *E. histolytica,* when the tetragena type is present. It is noted that when there is a large, spherical, solid-appearing karyosome there is little evidence of a linin network or of free chromatin within the nucleus, while the inner surface of the nuclear membrane is almost free from granules of chromatin; but when division of the karyosome occurs, this portion of the nucleus appears smaller and stains less intensely, while the linin network is more distinct, contains much chromatin in the form of small granules, and large clumps of this substance may be observed on the inner surface of the nuclear membrane. The large clumps of chromatin just mentioned are quite characteristic of this species, for I have never observed such large masses on the nuclear membranes of the pathogenic species.

Not infrequently specimens of *E. coli* are observed undergoing simple division, and in stained preparations it is possible to study this process from the initial division of the centriole to the complete division of the nucleus. Very frequently organisms are observed in which the karyosome consists of two deeply stained oval masses of chromatin situated near the center of the nucleus or near its periphery, these masses sometimes being connected by a dimly stained thread of chromatic substance. More frequently the masses are apparently separate and either oval or spindle-shaped, while the interval between them appears dimly granular; but I have never observed any evidences of an equatorial plate. Other organisms are observed in which the karyosome has disappeared and the inner surface of the nuclear membrane is covered with large, irregular clumps of chromatin, the remainder of the nucleus being filled with fine, dimly staining granules; and still others are observed containing two well-developed nuclei, each showing the large, spherical, deeply stained karyosome characteristic of the nucleus of this species before the changes prior to division occur.

Besides the reproduction of the organism by division, Schaudinn described, in the vegetative stage of development of *E. coli,* a process of schizogony, in which the parent entameba produced eight young entamebas. I confirmed this observation and Hartmann and Whitmore[28] have published beautiful illustrations of vegetative organisms containing as many as six nuclei. In my experience, this method of reproduction is very rarely observed, but I am convinced that it occurs and that the organisms in which I have observed it were not in the cystic stage of

development. The process is initiated by an enlargement of the nucleus and a considerable increase in the amount of chromatin within it, the karyosome being indistinguishable. Later the inner border of the nuclear membrane shows hemispheral masses of chromatin, arranged in a very regular manner, the remainder of the nucleus being entirely free of this substance. Organisms are observed containing from two to eight nuclei, each nucleus having a distinct nuclear membrane and a small, dot-like or granular appearing karyosome. This method of reproduction has been questioned by several observers, notably James, but, in my opinion, no other interpretation can be placed on these organisms.

Degenerative forms of *E. coli* are frequently encountered during the vegetative stage of development, which may cause confusion in the diagnosis of the parasite. In such organisms the nucleus may be invisible or distorted in appearance, and the cytoplasm may contain chromidia, as in *E. histolytica*. These organisms should not be considered in making a diagnosis of the species of entameba present, for typical parasites will always be found in stained preparations if carefully searched for, and the technic of staining has been correct.

In some preparations entamebas are observed without a nucleus and free nuclei may be present in large numbers. These free nuclei stain well and are typical in structure, and the exact significance of their occurrence is still in question. This peculiar phenomenon was first called to my attention by James, who sent me preparations containing many examples of anucleate entamebas and free nuclei, and I have no explanation to offer of their presence, beyond the suggestion that possibly they may be due to some change brought about in fixing the preparation or in smearing it on the slide. I do not believe that the condition is due to degenerative changes, for the nuclei appear normal as do the anucleate entamebas.

The vegetative forms of *E. coli* occur most typically in diarrheal stools or stools rendered liquid by some cathartic and, as this parasite is not pathogenic in character, these forms are seldom observed in the normal formed stools of the host, only the precystic or cystic forms being found in semiformed or formed stools. For this reason I have found that the administration of a cathartic is essential if one desires to study the typical vegetative stage of *E. coli*, but inadvisable if one desires to study the precystic or cystic stage. It is unfortunate that the differentiation of *E. coli* is of the greatest practical importance in just the class of cases in which the vegetative forms occur, that is, in cases showing diarrhea, for the vegetative forms are much harder to differentiate from the vegetative forms of *E. histolytica* than are the cysts of the two species.

Morphology in the Pre-Cystic Stage.—In semifluid stools the precystic forms of *E. coli* are most frequently encountered. In this stage of development the parasite is reduced in size and resembles so closely the precystic stage of *E. histolytica* as in many instances to be almost indistinguishable.

In the living specimen, the shape is usually oval or spherical, but irregular if motility is present. The size varies from 8 to 15 microns, the average being about 12 microns. Motility is very sluggish, very rarely progressive, and generally consists in the sending out of small, blunt pseudopodia, which are immediately withdrawn, so that no progressive movement results. The cytoplasm appears more refractive than in the vegetative stage, and it is but rarely that any distinction can be made between the ectoplasm and endoplasm, although occasionally the endoplasm appears to be retracted and surrounded by a clear zone representing the ectoplasm. The nucleus is generally visible as a ring-like, refractive body, within which shining granules of chromatin may be observed. At this stage of development the cytoplasm does not contain much extraneous matter and vacuoles are less numerous, and generally entirely absent.

In stained preparations the parasites, in many instances, show but little structure, owing to the intensity with which they take the stain. In well-decolorized preparations, however, the cytoplasm appears granular and more or less reticulated, while the nucleus presents a deeply stained, rather thick nuclear membrane, and a distinct karyosome consisting of a deeply staining, spherical mass of chromatin. I have observed in the karyosome evidences of division as shown by thinning of the chromatin and a partial separation of the karyosome into two oval bodies, united by a dimly stained thread of chromatic substance. I have never seen a precystic *E. coli* with more than one nucleus, although sometimes it has required considerable care to differentiate cysts containing two nuclei from the precystic forms, in cases in which the cyst-wall was poorly defined. I believe that no reproduction occurs during this stage of development, and that the differences in appearance between the vegetative and precystic forms are, in the latter, entirely preparatory to encystment.

Morphology in the Cystic Stage.—The cysts of *E. coli* are characterized by their large size, when compared with *E. histolytica,* and when fully developed contain eight nuclei, although this number may be exceeded, as many as sixteen nuclei having been observed. The cysts are found most frequently in formed stools, although in patients suffering from diarrhea the cysts may sometimes be observed along with vegetative forms. This occurs only where the cycle of development has already reached the cystic stage before the stools became semifluid or fluid.

The living cysts of *E. coli* are spherical or oval in shape and refractive in appearance. The size varies considerably, cysts being observed measuring as much as 25 or 30 microns in diameter, and others as little as 10 microns, but the average diameter is about 15 microns.

The cyst-wall varies much in appearance with the age of the cyst, being barely distinguishable as a delicate line surrounding the organism at the beginning of encystment, but presenting a double outline when the cyst is fully developed, the membrane being of considerable thickness. Just prior to encystment the organism rids itself of any bacteria, crystals or other extraneous matter that it may contain and the cytoplasm assumes a distinctly alveolar appearance. As encystment progresses this alveolar structure is gradually replaced by a finely granular one, and in some of the cysts a large vacuole develops, filling nearly two-thirds of the cyst, the cytoplasm being collected into a granular band surrounding the vacuole, and containing one or two nuclei. When two nuclei are present they are often situated at opposite sides of the cyst, separated by the vacuole already mentioned.

In some of the younger cysts a few shining granules of chromidia may be observed, but these disappear before division of the nucleus begins, and I have never observed any of the large chromidial masses so characteristic of the cysts of *E. histolytica*.

The cysts may contain from one to as many as sixteen nuclei, the normal number, when the cyst is fully developed, being eight. In the living specimen these nuclei appear as ring-like, refractive bodies scattered through the cyst, and, on account of being situated at different levels in the cytoplasm, it is necessary to focus carefully in order to bring them all into view. The cysts that contain more than two nuclei very rarely show the large vacuole mentioned, this being most often observed during the primary division of the nucleus.

In specimens of *E. coli* stained with iron-hematoxylin or chlorid of iron-hematoxylin, the cystic membrane remains unstained, in most instances appearing as a hyaline band, of single or double outline, encircling the cyst. The cytoplasm appears alveolar in the young cysts, but in the older ones it generally is finely granular in appearance. In the cysts showing but one nucleus a large vacuola is often present and this may be found in cysts showing two distinct nuclei; but in the cysts containing from four to eight nuclei I have never observed the vacuole, so that it is evident that it disappears as nuclear division progresses. In the cysts containing the large vacuole, the nucleus, when single, is of large size and presents evidences of division, the karyosome being divided into two oval or spherical portions, connected by strands of chromatin, or entirely divided. In these nuclei the membrane is well stained, of considerable thickness, and not infrequently the nuclear chromatin, instead

of being collected into a distinct, dividing karyosome, appears to be divided into threads running from pole to pole of the elongated nucleus. In the cysts containing two nuclei and a large vacuole, the nuclear membrane of each nucleus is distinct, and there may be a well-defined karyosome consisting of a deeply stained mass of chromatin, or evidences of consisting of a deeply stained mass of chromatin, or evidences of division may be noted, the nuclear chromatin being arranged in more or less definite strands within the elongated nucleus. In the cysts that do not present the large vacuole, the nucleus, when single shows a definite karyosome, a well-marked nuclear membrane, and granules of chromatin scattered through it between the membrane and the karyosome. In such cysts the nucleus resembles closely that observed in the vegetative forms. In the cysts that contain two nuclei, however, evidence of division of these is often noted, the nuclei resembling those found in the cysts containing the large vacuole.

As nuclear division progresses in the cysts, the resulting nuclei become much reduced in size, are generally spherical in shape, and the elongation and arrangement of the chromatin into threads stretching from pole to pole is not so evident. In fact, many of these nuclei consist merely of a well-defined nuclear membrane, containing a loose mass of granules near the center, representing the karyosome. In many of them a considerable thickening of the nuclear membrane appears at one portion of the periphery, giving a peculiar cup-shaped appearance to the outline of the membrane.

In the cysts with more than eight nuclei the nuclei are still more reduced in size and consist simply of a thin nuclear membrane containing scattered chromatin granules, or a few granules situated at the center, representing the karyosome.

It is not unusual to observe cysts that are evidently undergoing degeneration, the cystic membrane being poorly defined and the entire cytoplasm of the cyst being filled with deeply stained granules, threads or masses of chromatin, the nucleus having disappeared, or, if present, being distorted in shape, while the nuclear structure is obscured or atypical in character. Sometimes these degenerate cysts contain one or more compact, quite large masses of chromatin, resembling the chromidial masses so characteristic of the cyst of *E. histolytica,* but the absence of a definite nucleus, and the smaller size, and irregular shape of these masses should serve to distinguish the cysts containing them from those of the latter parasite.

Resistance to Physical Conditions and Chemical Agencies.—Practically no work has been done on the resistance of *E. coli* to physical conditions or to chemicals, but reasoning from analogy, it is probable that this species follows very closely *E. histolytica* in this respect.

Relation to Disease.—E. coli is frequently observed in the feces in both health and disease, and although it is probably world-wide in its distribution, the percentage of persons infected varies greatly in different localities, being highest in the tropics and subtropics, and lowest in the temperate zones.

This species has been often mistaken for the pathogenic *E. histolytica,* and for this reason it is important to know the percentage of persons that may be expected to show it in various parts of the world. Schaudinn[1] found it present in 50 per cent. of healthy persons in West Prussia; in 20 per cent. in Berlin, and in 66 per cent. of healthy people examined on the shores of the Adriatic Sea. In 1905, I[32] examined over 200 healthy American soldiers at San Francisco, recruited from all parts of the United States, and found that 65 per cent. of them showed *E. coli* in their stools after a dose of magnesium sulphate. Later, in conjunction with Ashburn,[33] I found 71 per cent. of infections with this parasite among 107 soldiers examined at Manila, Philippine Islands. Vedder[48] found 50 per cent. of healthy American soldiers infected in the Philippines, and 75 per cent. of the native troops. Vedder's observations were made on 50 American soldiers and the same number of Philippine scouts. Sistrunk[35] found *E. coli* in eleven out of 145 patients examined at St. Mary's Hospital, Rochester, Minn., the patients suffering from diseases other than dysentery, while Rosenberger,[36] at the Philadelphia General Hospital, found 105 out of 106 insane patients examined infected with this parasite. In 1905, I[37] examined the stools of 250 patients suffering from diseases other than dysentery, at the U. S. Army General Hospital in San Francisco, and found that 49 per cent. of them showed *E. coli* in their feces.

From these data it is evident how widespread is infection with this parasite, and in what a large proportion of persons in both health and disease it may be demonstrated. It is also evident that if one were to rely only on the presence of an entameba in the feces in the diagnosis of dysentery, without reference to the species present, every one of these

32. Craig, Charles F.: Observations Upon Amebas Infecting the Human Intestine, Am. Med., 1905, ix, 854, 897 and 937.

33. Ashburn, P. M., and Craig, C. F.: The Work of the Army Board for the Study of Tropical Diseases in the Philippine Islands, Mil. Surg., 1907, xxi, 38, 140, 220, 336 and 527.

34. Vedder, E. B.: An Examination of the Stools of One Hundred Healthy Individuals with Special Reference to the Presence of Entamoeba Coli., Jour. Am. Med. Assn., 1906, xlvi, 870.

35. Sistrunk, W. E.: Intestinal Parasites Found in Individuals Residing in the Northwest, Jour. Am. Med. Assn., 1911, lvii, 1507.

36. Rosenberger, R. C.: Proc. Path. Soc., Philadelphia, 1912, v, 27.

37. Craig, Charles F.: Observation Upon Amebas Infecting the Human Intestine, Am. Med., 1905, ix (reference 103); also The Parasitic Amoebae of Man, Philadelphia, 1911.

persons would have been diagnosed as suffering from a latent entamebic dysentery, and this was exactly what was done prior to the work of Schaudinn and his followers.

It is also evident that the question of the pathogenicity of *E. coli* becomes a most important one, and it has been absolutely proved that this species of entameba is harmless. While much work was accomplished prior to Schaudinn's researches, and there was a well-grounded belief that certain species of entamebas were non-pathogenic, it was not until Schaudinn's paper appeared that there were sufficient data to enable one to differentiate the species of entamebas. Schaudinn proved on his own person that *E. coli* is a harmless parasite, capable of living in the intestine, but incapable of penetrating the mucous membrane or causing the lesions or symptoms of dysentery.

In 1905, while studying entamebas at the Army General Hospital, in San Francisco, I tried repeatedly to produce dysentery in young kittens with *E. coli*, both by injecting into the rectum material containing entamebas and by feeding similar material containing both the vegetative and encysted forms, but I was never able to produce the least symptom of dysentery, although my experiments with material containing *E. histolytica* were successful. My experiments were confirmed by numerous investigators and the recent contribution of Walker and Sellards[21] furnishes the final proof of the harmless nature of this parasite.

These investigators fed twenty men with material containing the cysts of *E. coli*, of which number seventeen became parasitized at the first feeding. None of these men developed dysentery, although Walker and Sellards were successful in producing dysentery in some of the men to whom they fed material containing *E. histolytica*. The period of incubation varied from one to eleven days in the case of *E. coli*, with an average of 4.7 days.

This parasite may persist in the intestine of man for a very long period of time, and I have personal knowledge of infections with *E. coli* that have lasted for from three to ten years, during which time no symptoms of dysentery have ever developed.

Differential Diagnosis.—Owing to the large proportion of infection with *E. coli* in regions where entamebic dysentery occurs, and the ease with which this species may be confused with the pathogenic *E. histolytica*, it is most important that the two species be differentiated. This may be best done in the living condition, from a practical point of view, but such a diagnosis should, if possible, be confirmed by preparations wet-fixed and stained by one of the hematoxylin methods, preferably Heidenhain's or Mallory's iron-hematoxylin method.

In the vegetative stage of development, in living preparations, *E. coli* may be differentiated from *E. histolytica* by its sluggish motility, poor distinction between the ectoplasm and endoplasm, large, clearly visible nucleus, and the absence of red blood-corpuscles within the endoplasm, when the stools contain blood. In stained preparations, in this stage of development, the parasite is distinguished from *E. histolytica* by the thick nuclear membrane, large granules and clumps of chromatin on the inner side of the nuclear membrane, the large, compact karyosome, and the absence of red blood-corpuscles within the endoplasm. In the precystic stage of development, in both living and stained preparations, it is exceedingly difficult and often impossible to differentiate this species from *E. histolytica,* but there are almost always vegetative or cystic forms present on which such a differentiation may be based.

In the cystic stage of development, *E. coli* may be differentiated from *E. histolytica,* in the living condition by the large size of the cyst, its thicker wall, the absence of large chromidial bodies within it, and the presence of eight or more nuclei, only four nuclei being present in the cyst of *E. histolytica.* The same differential points are of value in the stained preparations.

OBSERVATIONS ON CRAIGIA HOMINIS, CRAIG, 1906, EMEND. CALKINS, 1912

In 1906, I observed this organism in the feces of a Filipino suffering from chronic diarrhea, and the same parasite was afterwards observed in the feces of five other Filipinos. I published my observations in the same year,[38] giving the parasite the name *Paramoeba hominis,* placing it in the genus *Paramoeba* founded by Schaudinn to include a marine ameba, because of its general resemblance to *P. eilhardi,* the type-species of the genus. Doflein,[39] suggested that this parasite should be placed in a new genus, as he did not consider that it was a true parameba and, in 1912, Calkins[9] established for it the genus *Craigia.*

Regarding the parasite Calkins says:

> Craig, 1906, observed a small amoeba in the human intestine in six cases of chronic diarrhoea. He named it *Paramoeba hominis* because of the presence of an accessory body which he compared with the *Nebenkörper* of *Paramoeba eilhardi* and the formation of flagellated swarmers. The identity of the accessory body with the Nebenkörper is questionable while the single flagellum of the swarmers is sufficient to throw it out of the genus named by Schaudinn. For these reasons I propose to give a new generic name —*Craigia*—after its discoverer.

38. Craig, Charles F.: A New Intestinal Parasite of Man, Am. Jour. Med. Sc., n. s., 1906, cxxxii, 214; also "The Parasitic Amoebae of Man," Philadelphia, 1911, p. 215.

39. Doflein, F.: Lehrbuch der Protozoenkunde, Ed. 3, 1911, p. 603.

Further study of this parasite has convinced me that Professor Calkins' criticisms regarding the generic position are just, and I am in agreement with him that it should be placed in a new genus.

But very little is known regarding the distribution of this parasite. At the time that I described it I had not encountered any infections except in native Filipinos, but in 1908, at Fort Leavenworth, I observed three infections with the organism in American soldiers who had recently returned from the Philippine Islands. During the past four years I have been so situated that I have had no opportunity of continuing observations on the parasite, but recently I have been informed by Dr. W. D. Bretz that he has observed a case of infection at Memphis, Tenn., so that it is evident that the parasite also occurs in the United States. Indeed, I have always been convinced that a careful study of the feces in cases of chronic diarrhea would demonstrate that *C. hominis* has a wide distribution and is a comparatively common parasite, being mistaken in its ameboid stage of development for *E. coli,* and in its flagellate stage for trichomonads or cercomonads.

Morphology.—C. hominis passes through both an amebic and a flagellate stage of development, and it is quite easy to follow the entire life cycle by making frequent examinations of the feces from day to day, while the symptoms of diarrhea are present. There are still certain points regarding the various phases of the cycle that are undiscovered or undecided, but I have been able to study each stage of development, including encystment, the formation of the swarmers, the process of longitudinal division of the flagellates, and the development of the amebic from the flagellate stage. During each of these phases of development the morphology of the parasite varies so that it is necessary to consider each separately in our description.

The life cycle of the organism may be briefly described as follows: Beginning with the amebic stage, the parasite reproduces by simple division for a number of generations (as long as conditions are favorable) as a typical ameba, at the end of which time it encysts, and within the cyst there develop numerous small swarmers, which finally escape, develop each a single flagellum, and reproduce by longitudinal division for several generations, after which the flagellum disappears, the organism develops ameboid motion, and again begins the amebic stage of development.

For convenience of description the morphology in the amebic stage, the cystic stage and the flagellate stage of development will be considered separately.

Morphology in Amebic Stage.—The living parasite varies considerably in size in this stage of development, organisms measuring as little as 10 microns being sometimes observed, while others measure as much

as 25 microns in diameter; but the average measurement in diameter at this stage is about 18 microns. The resting amebas are round or oval, but when moving they are very irregular, and several pseudopodia may be projected from the periphery at the same time. Motility in the fully developed amebas is very active, progressive movement is well marked, and there is a sharp distinction between the ectoplasm and endoplasm, the latter being much more refractive, the ectoplasm being thin and veil-like in appearance. In the motionless organism there is no distinction between the ectoplasm and endoplasm, and this is also true of the smaller parasites, in which the motility is very sluggish. In the living organisms a nucleus can nearly always be distinguished appearing as a refractive, spherical body, surrounded by a thick and more refractive nuclear membrane of granular consistence. Often this membrane appears to be composed of refractile rods arranged end to end around the periphery of the nuclear substance. In some of the fully grown amebic forms a brightly glistening oval body, minute in size, may be observed lying near or in contact with the nucleus, appearing much like the blepharoplast of a trypanosome or of the flagellate stage of a Leishmania. This is the body that, in my original description, I considered corresponded to the *Nebenkörper* of *Paramoeba eilhardi*, as described by Schaudinn.

The ectoplasm is always to be distinguished from the endoplasm in the larger organisms when the parasites are in motion, but when motility is very sluggish in the smallest parasites the distinction is lost, although even in the smallest examples of *C. hominis* the ectoplasm is well defined if motility is at all active. The ectoplasm appears homogeneous, while the endoplasm is reticular in structure, often giving the appearance of being filled with vacuoles, and it may contain bacteria, crystals and red blood-corpuscles. I have not observed vacuoles in the cytoplasm of this species but have no doubt that under favorable conditions they do occur.

In stained preparations the ectoplasm and endoplasm are not differentiated, the cytoplasm appearing reticulated and containing bacteria, crystals, and red blood-cells in many instances. The nucleus stains intensely, the nuclear membrane being thick and well defined, while the karyosome is large, and separated from the nuclear membrane by an unstained area devoid of chromatin. The karyosome stains uniformly throughout, a centriole being absent. The nuclear morphology is very similar to that of the genus *Vahlkampfia*, the only difference so far as I have been able to observe, being that this species has a smaller karyosome.

The accessory nuclear body is found only in some of the larger organisms. It appears as a deep black dot or thick, oval rod, lying near or almost in contact with the nucleus.

Organisms undergoing division are observed in stained preparations, and it is apparent the division first occurs in the accessory body, for this body may be seen divided before complete division of the nucleus has occurred. During division the chromatin of the nucleus becomes arranged in strands running from pole to pole of the elongated nucleus, and in some instances traces of an equatorial plate may be observed. Often the chromatin is collected into two cup-shaped masses at the poles of the nucleus and the space between is filled with fine granules without any definite arrangement. Organisms are observed containing two distinct nuclei, each possessing the structure found in the nucleus of adult *Craigia* before division. I have never observed any evidences of schizogony in this species.

Morphology in the Cystic Stage.—When alive the cysts of *C. hominis* are slightly smaller than the adult vegetative forms, measuring, on the average, about 15 microns in diameter, but there is a well-marked precystic stage, in which ameboid motility is lost, the organisms contract and expel any extraneous matter that they may contain, and then begin to rotate rapidly without changing position in the material containing them. This process of rotation may last for as long as one hour, but generally ceases in about fifteen minutes, when it will be noted that a distinct cyst-wall has been formed, possessing a double outline, while the nucleus and accessory body may still be differentiated within the cytoplasm. As development progresses the nucleus and accessory body disappear and the cyst becomes filled with refractive, spherical granules, which eventually become the young flagellates. When fully developed, the cysts of *Craigia hominis* appear as refractive, spherical bodies, surrounded by a well-defined cyst-wall, and containing numerous very refractive spherical bodies.

The cysts stain so intensely, evidently because of the richness of chromatin, that it is impossible to make out much in the way of morphology in such specimens. In specimens that have been decolorized for a long time one may sometimes see a collection of small ring-like bodies, evidently representing the nuclei of the swarmers, but no fine morphological details can be distinguished.

Morphology in the Flagellate Stage.—I have never observed the actual liberation of the flagellates from the cyst, but groups of these organisms are observed arranged in a spherical mass, and surrounded by granular detritus, evidently the remains of the cyst.

The young flagellates appear at first as spherical bodies, measuring from 3 to 6 microns in diameter, having a finely granular cytoplasm, and a poorly defined refractive nucleus. If these young forms be watched a single, very delicate flagellum appears at one portion of the periphery, and as soon as this occurs, the organism moves forward in a

jerky manner, propelled by the flagellum. Contrary to my original belief that the flagellum is situated posteriorly, I now believe it is situated anteriorly, for motion is observed in both directions, and in the fully developed flagellate motion is more often observed in the direction of the flagellum.

When fully developed the flagellates measure from 10 to 20 microns in diameter, are spherical in shape, slightly elongated at the portion from which springs the flagellum, and composed of a finely granular cytoplasm in which lies a small, but well-defined nucleus. In some of the flagellates the accessory nuclear body may be distinguished as a brightly refractive dot or rod near the nucleus. The latter is spherical in shape and has a well-defined, delicate nuclear membrane composed of refractile granules.

Reproduction in this stage of development occurs by longtiudinal division, and organisms are observed in which every stage of this process may be demonstrated. I have not been able to ascertain if the accessory nuclear body divides before the nucleus does, but parasites are observed containing two accessory bodies, two nuclei, and showing commencement of division in the flagellum, while others are observed in which the division of the two flagellates is almost complete, including the flagellum.

In stained preparations the cytoplasm of the flagellates stains very intensely and it is difficult to make out the structural details of the nucleus. The nuclear membrane is thinner than in the amebic stage of existence, and is apparently composed of minute granules; the karyosome is comparatively large and is surrounded by an unstained area as in the vegetative forms. In fact, the nucleus in the flagellate organisms is quite similar to that in the vegetative stage and markedly resembles that of the genus *Vahlkampfia*. The accessory nuclear body stains as a black dot or rod. The flagellum stains poorly; the most careful focusing is required to demonstrate it in most organisms. In the dividing forms two accessory nuclear bodies may sometimes be distinguished, while the nucleus shows evidences of a primitive form of mitosis, the karyosome dividing and the chromatin collecting at the poles of the elongated nucleus.

Relation to Disease.—There is no evidence that *Craigia hominis* is a pathogenic parasite, beyond the fact that I have found it present only in patients suffering from diarrhea, and that treatment causing it to disappear also resulted in the cure of the disease. I have been unable to produce infection in kittens with material containing this parasite, and there are no reports of pathologic lesions caused by it, so that at present, while I am of the opinion that it is capable of causing a form of chronic diarrhea, its pathogenicity is still unproved.

Differential Diagnosis.—In my original contribution I called attention to the likelihood of confusing this parasite with other entamebas and with trichomonads and cercomonads, and described in detail the differential points, but despite this, Hartmann[40] has suggested that I mistook certain stages in the development of *E. coli* and *Trichomonas intestinalis* for a new species of intestinal entameba. The absurdity of such a statement must be apparent to any one who has studied the organisms mentioned, and it may be stated that only one entirely inexperienced in the study of the protozoa of the intestine could make such a mistake.

C. hominis is distinguished from either *E. histolytica* or *E. coli* by its small size, the character of the nucleus, the presence of an accessory nuclear body, the structure of the cysts, and the production of swarmers within the cysts. From *T. intestinalis* it is distinguished in the flagellate stage by its larger size, the absence of an undulating membrane, the single flagellum, and the spherical shape. In the amebic stage it is distinguished from the resting forms of *T. intestinalis*, which sometimes possess a very limited degree of ameboid motion, by the larger size and the active progressive motion, which is never observed in trichomonads.

The differential points mentioned also serve to distinguish *C. hominis* from any entameba or intestinal flagellate hitherto described.

It is probable that careful observation will demonstrate that many of the instances of chronic diarrhea now accredited to *T. intestinalis* are really due to this parasite, but this will not be determined until there is a more general resort to the microscopical examination of the feces in clinical diagnosis.

OBSERVATIONS ON VAHLKAMPFIA LOBOSPINOSA, CRAIG, 1912, EMEND. 1913

The free-living amebas belonging to the genus *Vahlkampfia* are of great interest because of their frequent occurrence in material from cases of entamebic dysentery and the fact that they can be easily cultivated on various culture mediums. The genus also contains parasitic species found in some of the lower animals, and Wenyon,[41] Chatton,[6] James[42] and others have observed vegetative forms of *Vahlkampfia* in feces from the human intestine, so that it appears to be proved that some species of this genus are able to live a parasitic existence in man, but in every instance these organisms were easily distinguished

40. Hartmann, M.: Article on "Die Dysenterie Amoeben," in von Prowazek's Handbuch d. Path. Protozoen, 1912, i, 51.

41. Wenyon, C. M.: Intestinal Amebiasis, Brit. Med. Jour., 1913, 2495, p. 1244.

42. James, W. M.: Personal communication.

from *E. histolytica* and *E. coli* and presented the typical limax structure ordinarily observed in cultures.

As these amebas occur so frequently in cultures made from feces or material from liver abscesses in cases of entamebic dysentery, almost always as contaminations, the morphology of a typical example of the genus will be described. This organism, which I named *Amoeba lobospinosa* in 1912,[43] but which really belongs to the genus *Vahlkampfia*—and for which I now suggest the name *Vahlkampfia lobospinosa*—has a most interesting history. It was originally cultivated from the feces of a patient with entamebic dysentery by Dr. Musgrave, in Manila, who described it as Ameba 11524 of his series. The culture with which I have worked is descended from a culture he sent to the Army Medical Museum in 1905. I have also worked, so far as morphology and life history is concerned, with two other cultures of the same organism, which were sent to the museum by the Board for the Study of Tropical Diseases, in Manila, in 1908. This organism has thus been cultivated on Musgrave and Clegg's agar-medium for a period of nearly eight years, and during this time the morphology has not changed, but has remained that of a typical *Vahlkampfia*. In a recent contribution Calkins and Williams[5] have shown that on certain mediums this organism changes considerably in morphology and closely resembles, as they claim, *E. histolytica* or *E. coli*, and their work will be referred to later.

On the medium of Musgrave and Clegg, *Vahlkampfia lobospinosa* presents two well-defined stages of development, the vegetative and the cystic, and the morphology of the organism varies in each stage.

Morphology in Vegetative Stage.—The size of the organism varies exceedingly, measurements of from 8 to 25 microns in diameter being frequently observed, but the average diameter is about 16 microns. The shape, when motionless, is oval or spherical, but when moving the organisms are very irregular in shape. Motility is sluggish and progressive motion not very marked; the pseudopodia are of two varieties—short and blunt, and longer and spinose. The lobose pseudopodia are present when the organisms are actively motile, but the spinose pseudopodia are more frequently observed when motility is absent or very sluggish. The spinose pseudopodia are extruded from the periphery of the ameba very rapidly and as rapidly withdrawn, so that it is often difficult to distinguish them. Both lobose and spinose pseudopodia may be present at the same time.

The nucleus is well defined, appearing in the living organism as a spherical, refractive mass surrounded by a narrow hyaline area, while the nuclear membrane is indistinct. In most of the vegetative forms

43. Craig, Charles F.: Observations Upon the Morphology of Parasitic and Cultural Amoebae, Jour. Med. Research, 1912, xxi, 1.

a single, contractile vacuole is present, which contracts once in about fifteen seconds, and which, when most clearly defined, is situated near the nucleus but travels toward the periphery before it contracts. In most of the organisms the contractile vacuole appears to be situated at the periphery when contraction occurs.

In stained specimens the distinction between the ectoplasm and endoplasm, which is quite marked in the living condition, is lost, and the entire cytoplasm stains a brownish gray or gray, appears reticular in structure, and composed of granular material. The nucleus is characteristic in structure, being composed of a large karyosome, filling at least two-thirds of the entire nucleus, surrounded by a clear unstained area, which, in turn, is surrounded by a zone of fine chromatin granules forming a poorly stained nuclear membrane. The karyosome stains intensely and, unless the preparations are well differentiated, does not show a centriole, but in well-stained and decolorized preparations a centriole may be observed within the karyosome, consisting of one or two almost black dots. The unstained area surrounding the karyosome is devoid of chromatin granules and no traces of a linin network can be distinguished in this area. In many of the vegetative forms the nuclear membrane cannot be distinguished from the cytoplasm, but it may appear well stained and then consists of a rather thick membrane, on the inner side of which there is a zone of fine chromatin granules.

The cytoplasm contains many bacteria and often the single vacuole is well differentiated, together with unstained areas demonstrating the reticular nature of the protoplasm.

During the vegetative stage of development reproduction occurs by simple division, the nucleus often showing well-defined mitotic figures. Organisms are observed in which the karyosome as well as the entire nucleus is greatly lengthened, while evidences of division of the centriole are noted in well-differentiated preparations. A later stage in division is shown in organisms having deeply stained crescentic bodies at each pole of the nucleus, connected by fine filaments of poorly stained material, the whole forming a well-defined spindle. An equatorial plate is not infrequently observed consisting of a band of deeply stained granules of chromatin stretching across the spindle midway between the polar bodies, and sometimes a distinct division of this plate into two rows of deeply stained granules may be observed. Still later the equatorial plate and the spindle disappear and the nucleus is divided into two portions, spherical in shape, each having a distinct karyosome and a poorly defined nuclear membrane. In this species the mitotic character of the division of the nucleus is well marked, much more so than it is in the intestinal entamebas.

Morphology in the Cystic Stage.—Vahlkampfia lobospinosa produces cysts whenever the cultural conditions are unfavorable to vegetative existence, and on the ordinary culture mediums the cysts are characteristic of this genus, only one daughter ameba being formed in each cyst. In size the cysts vary from 8 to 15 microns in diameter, the average being about 10 microns. The youngest cysts, when living, have a delicate cyst wall, of single outline, but the fully developed cysts show a thick wall, very refractive in character, and having a well-marked double outline. The cysts are spherical in shape so long as the medium is moist, but as drying occurs they contract unequally and irregular forms are produced, generally quadrilateral or pyramidal in shape. The old cysts often have a very thick wall which appears cracked or folded over in places, thus giving the cyst a very characteristic appearance. The cytoplasm of the young cysts appears finely granular but free from bacteria, and the single nucleus may be observed near the center of the cyst. In the older, living cysts the nucleus is often invisible and the cyst appears to be filled with minute refractive granules, the nature of which I have been unable to determine.

In stained preparations the cytoplasm appears granular and the nucleus presents the same structure that is observed in the vegetative forms before division occurs. I have never observed more than one nucleus in a cyst, nor have I ever observed any evidences of division of this nucleus, so that encystment in this species, so far as it is shown on ordinary culture mediums, is purely a protective process, as reproduction does not occur within the cyst.

In brief, the description given above answers to the appearance of *V. lobospinosa* as observed in cultures on Musgrave and Clegg's nutrient agar, but the recent work of Williams and Calkins[6] has shown that when this species is grown in pure culture on brain-tissue medium very marked variation occurs in its morphology from what occurs when it is grown, in conjunction with bacteria, on ordinary mediums. They have found that the contractile vacuole disappears from the vegetative forms, the nuclei resemble in structure the nuclei of the entamebas, and the cysts become multinucleate, from two to four nuclei being present in many of them. Williams and Calkins consider these variations as significant of the parasitic nature of the species, but are very conservative regarding their exact significance, saying:[6] "We do not wish, however, to lay too much stress upon these experimental results; conditions of the culture might be such as to bring about variations when these do not occur under conditions 'normal' to the organism." These observations are of the utmost interest as showing how profoundly a protozoan organism may be modified by unnatural cultural conditions, but there is no reason to believe that these modifications in any way

connect this typical free-living ameba with the entameba of the intestine, as *E. histolytica* or *E. coli,* for even though the structure of the nucleus is modified it is perfectly evident to anyone who has worked with the entamebas that none of the photomicrographs published with the paper of Williams and Calkins show amebas with a nucleus in the least like the nucleus of either *histolytica* or *coli,* while even in the drawn plate the nuclei are markedly dissimilar in most of the drawings. It is a fact that whenever a *Vahlkampfia* has been found parasitic in the human intestine, and this has occurred in a few cases, as mentioned, the morphology has been typical of the limax type of ameba and the forms observed by these authors have never been noted. As regards the relation of these free-living species to disease the observations of Walker and Sellards, hereafter considered, are conclusive.

Relation to Disease.—There is at present no evidence that any species belonging to the genus *Vahlkampfia* is capable of producing disease in man, and it is evident from the investigations of many authorities that, in most instances, the species of this genus are actually incapable of establishing themselves as parasites in man. They are all essentially free-living amebas, so far as their relation to man is concerned, and while a very few instances have been reported of infection with the vegetative forms of *Vahlkampfia,* the infection lasted but a short time and no symptoms or lesions were produced by the organism. Therefore, we are justified in believing that such instances are accidental, and while they may be interesting from the point of view of the evolution of parasitism in a species, at present they possess no practical importance to the clinician.

To consider all of the observations that go to prove the fact that the species of the genus *Vahlkampfia* are essentially free-living as regards their relation to man would be only to repeat, but the recent work of Walker and Sellards, in my opinion, proves conclusively that the ordinary cultural amebas bear no relation whatever to the intestinal entamebas, but are in nature free-living and incapable of existing as parasites in man.

Walker and Sellards[21] experimented on ten men by feeding them cultures representing thirteen different strains and eight species of free-living amebas isolated from the Manila water-supply and other non-parasitic sources, from the stools of healthy persons and persons suffering from diseases other than dysentery, and also from dysenteric stools. They found that when cultures of these amebas were ingested by the men the amebas could be recovered from their stools in culture during the first few days after feeding but not subsequently, and that the microscopical examination of such stools always resulted negatively. No symptoms of dysentery occurred in any of the men, although they

were observed for months, and as the result of their work the authors draw the following conclusions:

1. The cultivable amoebae are incapable of living parasitically in the intestinal tract of man.

2. The amoebae, when obtained in cultures from stools, intestinal contents, or liver-abscess pus, are derived either from cultural contaminations or from encysted amoebae which have been ingested with water or food and have passed unchanged through the intestinal tract.

3. The cultivable amoebae are non-pathogenic, and consequently play no rôle in the etiology of endemic tropical dysentery.

In the light of these conclusive experiments it is very evident that no amount of evidence based on mere changes in morphology or life cycle produced by growing free-living amebas on unnatural culture material can be considered as of the slightest value in connecting them with the true entamebas, but that at most these variations simply indicate the path by which the present species of entamebas reached their parasitic mode of existence. From the point of view of evolution the experiments of Williams and Calkins are most valuable and interesting, but they in no wise affect the present classification of the parasitic species. I believe that it is proved that the cultivable amebas cannot, in the vast majority of instances and under natural conditions, live as parasites in man, and that they are entirely distinct from the entamebas and bear no relation to the etiology of entamebic dysentery.

In conclusion I desire to state that the personal observations recorded in this contribution were made at the U. S. Army General Hospital, San Francisco, Cal.; the Division Hospital, Manila, P.I., while the writer was a member of the U. S. Army Board for the Study of Tropical Diseases in the Philippines; the Rockefeller Institute for Medical Research, New York; the Laboratory of the Army Medical School, Washington, D.C.; and the Department Laboratory, Central Department, U. S. Army, at Fort Leavenworth, Kansas. For the photomicrographs, of which Figs. 1 and 2 are reproductions, I am indebted to Capt. Arthur C. Christy, U. S. Army. They were made from preparations stained with iron-hematoxylin and fixed with Schaudinn's sublimate alcohol solution.

THE PHYSIOLOGIC DEGENERATION OF THE MESONEPHROS

WITH A CONSIDERATION OF ANTAGONISTIC ACTION OF ORGANS AND OF FUNCTIONAL MUTATION WITH MORPHOLOGICAL PERSISTENCE *

HORST OERTEL
LONDON, ENGLAND

I propose to draw attention to certain embryological processes and phenomena which appear to have escaped a more than cursory attention by the pathologist and, if a perusal of the literature and an inquiry among those able to judge lead me to suppose correctly, have received a rather inadequate consideration by anatomists and physiologists. The road, therefore, is difficult to travel, and the observations and considerations which are recorded below will appear necessarily fragmentary and incomplete. Indeed, the difficulty of obtaining suitable material and other more personal obstacles allow me to offer no more than an outline of the matter. That, however, seems warranted by the importance and the unfortunate neglect of the subject. Even in incomplete form it may, nevertheless, obtain the interest and future help of those who by position and experience are able to enlarge on the facts and elaborate the conclusions to be drawn therefrom.

Perhaps it may not be amiss to review briefly, as an introduction, our present knowledge and ideas regarding renal development in general and the mesonephros in particular.

I. INTRODUCTORY GENERAL PART[1]

It is of course impossible to present within the scope of this paper, which proposes to deal only with certain phases of nephric development, a thorough discussion of the whole complex and still disputable subject of the origin of the excretory system and the relations it may have to the original annelid nephric organ. These to the pathologist by no means unimportant questions, must be studied elsewhere.[2] I shall con-

* From the Gordon Pathological Laboratory of Guy's Hospital.
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1. The introductory general part was essentially written for those unacquainted with present views of renal development and of the position and structure of the mesonephros; others may turn immediately to the second special part.

2. Consult Felix, W.: The Development of the Urogenital Oragns, in Man-

fine myself to a sketch of those features a knowledge of which appears necessary for an intelligent understanding of the subject at issue.

The kidney of all amniotes discloses, as is well known, its phylogenetic travels much plainer than any other organ. By succession and partly coexistence appear three distinct excretory organs, pronephros, mesonephros and metanephros. The pronephros disappears rapidly, but the mesonephros, or wolffian body, acquires full development and a high degree of differentiation. In man, particularly, this rises to a better state of evolution than in some of the lower animals, in pigs it reaches an enormous size and recedes only in embryos of 10 cm., while in reptiles it continues through part of the postnatal life. Thus in lizards the mesonephros persists to the end of the first year and, according to Braun,[3] retrogression of the mesonephros does not commence until after the first hibernation. On the other hand, it happens that in the mouse the wolffian body develops only incompletely and is even devoid of all glomeruli.

It is, therefore, evident from the start that the development of the amniotic kidney is quite unlike the gradual, uniform development of other organs, but *serial,* for it appears that pronephros, mesonephros and metanephros are to a considerable extent at least independent organs, which take origin from closely adjoining and partly overlapping segments of the mesoderm, so that the pronephros develops most cranially, caudally to it the mesonephros and still more caudally the metanephros or permanent kidney. The exact genetic relation of these three organs has been, and still partly is, a disputed question. For our discussion it is sufficient to emphasize that a relation exists so far as they represent renal excretory organs patterned on one structural scheme, but of a growing complexity which is demanded by progressively higher grades of general development, and corresponds to various stages of animal evolution. As one is replaced by the other the predecessor appears thereafter by recapitulation, rudimentary or provisional. A certain

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ual of Embryology, edited by F. Keibel and F. P. Mall, Philadelphia and London, J. B. Lippincott Company, 1912.

Felix, W.: Die Entwicklung des Harnapparats (1904), in Hertwig's Handbuch der vergleichenden Entwicklungslehre der Wirbelthiere, Jena, G. Fischer, 1906.

Gaskell, W. H.: The Origin of Vertebrates, London, etc., Longmans, Green & Co., 1908.

Broman, Ivar: Normale und abnormale Entwicklung des Menschen, Wiesbaden, Bergmann, 1911.

Bonnet, R.: Lehrbuch der Entwicklungsgeschichte, Berlin, Paul Parey, 1907.

3. Braun: Das Urogenitalsystem der einheimischen Reptilien, Arbeiten aus dem zool.-zoot. Institut zu Würzburg, 1877, iv.

Schreiner, K. E.: Ueber die Entwicklung der Amnioten Niere, Ztschr. f. wissensch. Zool., 1902, lxxi, 1-189.

structural resemblance may, therefore, be noted in all three which in amniotes is necessarily closest between mesonephros and metanephros. In myxinoids the pronephros represents the permanent kidney. In fishes and amphibia it atrophies and is replaced by the better-organized mesonephros. Finally, in amniotes the mesonephros also undergoes retrogression and is replaced by the most highly organized metanephros as permanent kidney. One other and more special relation between these three organs must be noted: There is developed in connection with the pronephros an excretory duct, called the primary excretory or segmental duct, which takes origin from the thirteenth and fourteenth and possibly other caudal segments of the pronephric anlage. It penetrates early into the cloaca. From it descend directly the wolffian duct of the mesonephros and the ureter of the metanephros with their derivatives.

An understanding of the functional and structural characteristics of all three kidneys is much helped by tracing them through phylogenetic evolution. In the lowest unicellular forms of animal life the general surface of the cell serves as a medium for the discharge of waste products. But under certain conditions, probably increased energy, there appear contracting vacuoles in addition to others of alimentation and distribution. The contracting vacuoles have been regarded as the first evidences of an excretory system. Thus Rhumbler believes that certain excretory granules and crystalline enclosures which were first observed by Stein in 1859, and later thoroughly investigated by Bütschli, are discharged by pulsating vacuoles. The nature of these granules or crystals is held to be either uric acid (Griffiths, 1889) or an oxalate (Bütschli) or calcium phosphate (Schewiakoff).[4]

We see further in the lower metazoa, such as the turbellarins, a complex system of tubes which ramify in the tissues of the body and constitute an independent excretory organ.

The appearance of the coelom or body cavity marks the beginning of a definitely recognizable excretory system which was combined in all probability from the start, or at least very early, with the sexual apparatus. This combination persists to the human organism, in which a separation of urinary and sexual organs has only partly been accomplished. Both form a unit during human embryonic and fetal life, and even in the adult one channel serves both as terminal passage. At the earliest stages of evolution both were represented by the coelom walls which possessed the ability not only of elimination, but also of producing sexual cells, gonads (Gegenbaur[5]). Gradually the coelom developed ducts

4. Von Fürth, O.: Vergleichende chemische Physiologie der niederen Thiere, Jena, G. Fischer, 1903.

5. Gegenbaur, C.: Vergleichende Anatomie der Wirbelthiere, Leipsic, W. Engelmann, 1898.

for conveyance of the excretory products to the exterior. It is very probable that later a certain portion of the coelom wall became reserved for the sexual function and another for the excretory function.

Thus originated sexual glands and pronephros, the first definite excretory organ of vertebrates. Notwithstanding their appearance, the association of the two systems continues quite intimate throughout the whole series of vertebrates, and is necessarily most marked during early life.

In this persistent and peculiarly characteristic interchanging relationship of excretory and sexual apparatus rest, as will appear, important factors which seem to enter into the developmental fate of both.

It has been stated that the structural organization of the three excretory glands is based on one scheme. This is dictated by their two main functions. These are (if we neglect for the time certain metabolic activities which acquire importance only in higher animals):

1. To remove useless or dangerous metabolic or foreign substances from the body (excretion).

2. To allow permeation and elimination of superfluous water from the body fluids (filtration).

Both combined maintain constancy in the composition of the body-fluids and the blood. It is very probable that the most primitive urinary organ, the coelom, or body cavity, was able to carry on both functions. Each coelom cell possessed excretory activity, and the coelom wall as a whole formed a membrane permeable for water. With growing complexity of differentiation it became necessary to specialize these functions in particular parts of the coelom cavity. Consequently that portion of the coelom wall which was richly provided with blood-vessels assumed filtrative function by the formation of the external glomerulus. This consisted only of an invagination of blood-vessels into the body cavity. On the other hand, the transverse ducts remained reserved for the more specific excretory function. This constituted the pronephric period. During later stages of development the filtration apparatus of the external glomerulus, originally quite independent, was incorporated in the kidney and quantitatively modified to adapt itself to the new environment and demands. Thus originated the malpighian corpuscles of the mesonephros and later of the metanephros. This union created an organ which again combined, as did the coelom, filtrative with excretory functions, but in a much higher degree of efficiency. At this point all communication between kidney tubules and coelom disappeared, and the latter retained only the mechanical function of separating movable organs from each other and the body walls.

The fact that the kidney of higher animals represents a union of originally independent units is of some interest in relation to its func-

tional activities, for it is likely that both units have largely retained their chief original purposes, the one of filtration, the other of secretion and excretion. In this connection it is interesting to recall that with progressing evolution the tubular secretory structures become not only more numerous but also more complex, and that their cells undergo various processes of differentiation. They show, therefore, important qualitative tissue changes. It is different with the glomerulus. From the one external glomerulus a step to many is made in the mesonephros and a further step to a still greater number in the metanephros. But the glomerular structure, unlike the tubules, remains essentially the same throughout evolution. One would conclude that glomerular function, although quantitatively enormously increased, has not undergone marked qualitative changes.

To recapitulate and sum up: All three kidneys are evolutionary stages of an organ series. They have in common secretory tubules opening into an excretory duct, but their structures arise from different segmental portions of the mesodermal plate, and, therefore, are not identical or evolutionary periods of one organ. At first, during the period of tubular formation from the cranial segments, the tubules are simple and retain segmental arrangement. A glomerulus lies outside and independent to the secretory portion (pronephros). Later, when formed in the dorsal and lumbar regions, tubules appear in greater number and become more complex, and glomeruli appear and are incorporated and connected to the tubules in a fully developed renal organ (mesonephros). In the last stage of sacral development the tubules assume greater complexity and number with a corresponding increase of glomeruli, and they first group themselves around and then unite with an outgrowth from the wolffian duct the ureter and its derivatives, all of which are the direct descendants of the pronephric primary excretory duct (metanephros).

This short, rather crude sketch may perhaps suffice to recall to mind the position which the mesonephros occupies in genetic and individual history, and we may now briefly review its structure and organization.

Attention has been drawn to the fact that the mesonephric tubules develop from those stalks of the primitive mesodermal segments which lie caudally from the pronephros. In mammals these form early a blastema or cell cord which gives rise in its cranial portion to the parenchyma of the mesonephros and later in its caudal portion to the parenchyma of the metanephros. This blastema is commonly spoken of as the nephrogenic cord, and its appearance terminates the existence of the critical period of individual segment stalks and inaugurates the possibility of a greater number of tubules of growing complexity. These tubules empty into the primary excretory duct, which now becomes the wolffian duct.

Recent investigations have settled the long dispute about the manner of kidney development in favor of a dualistic origin, according to which one anlage gives rise to the secretory, and another to the excretory part. The mesonephros, therefore, like the metanephros, later is formed by a union of parenchyma; that is, glomeruli and secretory tubules, derived from the nephrogenic cord with an outgrowth from the wolffian duct; that is, the collecting tubules. In the metanephros the parenchyma is joined to the pelvis of the kidney by the collecting tubules, both of the latter representing an outgrowth of the original ureter and termed the ureteric tree.

In appearance, structure and organization the mesonephros, therefore, bears such a striking resemblance to the permanent kidney that it may justly be regarded as an abbreviated metanephros.

Depending on its mesodermal derivation, the mature mesonephros is situated anteriorly to the position occupied later by the metanephros; that is, the lower dorsal and upper lumbar or hepatic regions. In relation to the embryo it is large and presents in shape a broad, slightly curved mound or ridge. Its malpighian corpuscles are similar to those of the permanent kidney and show well-developed glomeruli filled with blood and occupying the whole of the capsule. The latter is plainly lined by rather high epithelium. Marked differences exist between the convoluted and collecting tubules. The convoluted tubules bear, except for number, a striking resemblance to those of the metanephros, more particularly in regard to their lining epithelium, which present the general characters of all renal secretory epithelium in convoluted tubules. Mihálkovics[6] describes these cells as high, cylindrical (from 0.01 to 0.02 mm.), of yellowish, poorly staining protoplasm and distinctly granular. But Winiwarter and Sainmont[7] speak of them as cuboid epithelium, which I think is more correct for the intact undegenerated cells. Individually, the cells are sometimes poorly outlined so that, according to Mihálkovics, this epithelium appears in sections frequently like a nucleated yellowish syncytium. The nuclei are relatively large, round and rich in chromatin, and possess, according to Winiwarter and Sainmont, a large nucleolus. In early stages they show frequent mitoses. Mihálkovics describes irregular, colorless, thread-like processes (cilia) extending from the free cell ends into the lumen of the tubules, in which they seem to be continuous with a network which fills the wide lumen. He interprets these pictures as evidences of

6. Mihálkovics, G. Von: Untersuchungen über die Entwicklung des Harn und Geschlechtsapparats der Amnioten, Internat. Monatsschr. f. Anat. u. Histol., 1885, ii, p. 41 and p. 65.

7. Winiwarter, H. Von, and Sainmont, E.: Nouvelles recherches sur l'ovogénèse et l'organogénèse de l'ovaire des mammifères (chat), Arch. de biol., 1909, xxiv, 373. Involution du corps de Wolff.

glandular secretion. Winiwarter and Sainmont also observed the discharge of hyaline globules from these cells which they regard as secretion. I have been unable in the material at my command to discover similar structures except in degenerative processes. As, however, fixation and freshness as well as hardening and staining methods materially influence such details, I do not feel able to deny their existence. Whether or not they really represent products of a secretory activity is, of course, open to question.

In the straight and narrow collecting tubules which can be distinguished as easily from the convoluted ones as in the metanephros, the lining epithelium is low (from 0.007 to 0.008 mm., Mihálkovics), closely set, very distinct, clear, with smaller nuclei and resembles the lining cells of the wolffian duct, indicating a close relation of that tubular portion to the excretory duct.

The whole mesonephros is enveloped by a capsule composed in the earlier stages of development by several loosely arranged and cellular layers; in the mature organ, however, the capsule shrinks, and its cellular contents are lost. The parenchymatous or cortical part shows early many undifferentiated blastemic cells lying in a loose, thin stroma. As the tubules develop, these diminish, and in the fully developed mature mesonephros practically no interstitial cellular tissue is to be seen between the tubules of the cortex; but these lie in close proximity, separated only by thin but distinct basement membranes and intertubular capillaries. A thin, loose stroma is retained around the malpighian corpuscles. This is more abundant toward junction with the wolffian duct.

If, then, as will be apparent from the foregoing description, there exists an essential similarity in the organization of the mesonephros and metanephros, there are also, regardless of details, two very important differences, first in circulation, secondly in the connection with the sexual gland. These must, therefore, be shortly described.

The mesonephros enjoys a very rich and peculiar arrangement of its blood-supply. It receives in the first place a large number of arteries from the lateral aortic side. According to Bonnet,[2] there are no less than twenty in the fully-developed human mesonephros, but of even greater abundance is its venous portal circulation (Felix). In order to understand this properly one must recall that the mesonephros lies between the vena cardinalis posterior on the dorsal side and the vena subcardinalis on the ventral side. Both veins are connected at its caudal border with the venae caudalis and ischiadica. Early in mesonephric development numerous branches begin to form between the posterior cardinal and subcardinal veins, traversing the mesonephros. From the beginning the vena cardinalis posterior opens into the duct of Cuvier and through this into the sinus venosus of the heart, while the vena subcardinalis acquires

connection with the heart through the hepatic vein (Felix). We shall have reason to refer later to these peculiar circulatory conditions.

The second peculiarity of the mesonephros — its intimate connection with the sexual gland — will be referred to here but briefly, for it will form the subject of a later discussion. At a variable, but very early stage of mesonephric development, a narrow strip of mesothelium extending along the medial surface becomes prominent and cellular, forming the genital tubercle. These cells are later arranged into layers and become definitely differentiated and grouped. The whole mesothelial band is known as germinal epithelium and gives rise to the sexual glands, testis or ovary. A connecting band remains and is known, respectively, as mesorchium or mesovarium.

Lastly, the time over which mesonephric development extends is not uniform in amniotes, even in the mammals, in which it remains a purely embryonic organ. Traces of an early original segmental arrangement have been observed up to human embryos, but the segment stalks fuse rapidly to the nephrogenic cord, so that the early differentiation of the mesoderm is, in mammals at least, much abbreviated.

The time of appearance of the first mesonephric tubules varies. It has been tabulated by Felix, as follows: In the rabbit from the ninth to the tenth day (Köllicker, 1879) 245 hours after fertilization in embryos of from 5 to 5.5 mm. greatest length; a dog, eighteen days, four hours after coition showed from sixteen to twenty primary segment pairs and from eight to seventeen anlagen (Bonnet, 1887). In the pig the first mesonephric anlage is found fourteen days, nine hours after coition in embryos of from 5 to 5.3 mm. greatest length in ten or eleven primary segment pairs; in man the first tubules appear in embryos between 2.6 to 3 mm. greatest length (His, 1880; Keibel, 1896). The mesonephros is reported as complete in the guinea-pig on the twenty-third day after the last litter (Weber, 1897); in rabbits between 18 and 20 mm. length (Mihálkovics), or on the sixteenth day of development (Egl, 1876); in sheep of from 18 to 20 mm. length (Weber, 1897); in pigs of from 6 to 7 cm. length (Keibel, 1897; Neuhauser, 1903); in human embryos of 7 mm. length (Weber, 1897; MacCallum, 1902), and in the mouse the development, as mentioned, remains incomplete and no glomeruli are formed (Weber, 1897). When the mesonephros has reached its caudal limit, no new caudal anlages are added in older embryos, and a degenerative and destructive process becomes generally manifest which annihilates the organ with the exception of those anterior and middle parts which enter the services of the sexual organs. The manner, character and bearing of this process will form the subject of the following special part.

II. SPECIAL PART

The degeneration and ultimate fate of the mesonephros have been much discussed by anatomists from the developmental point of view, but the histological changes leading to its loss, their causes and possible bearing on certain problems of postnatal pathology have hardly received any attention. Nearly thirty years ago (1885) Mihálkovics[6] contributed in a classic work an investigation and description of the histological details. Nothing has been added since, with the exception of the recent work on the organogenesis of the cat's ovary (1909) by Winiwarter and Sainmont,[7] who describe the degenerative appearances in the mesonephric tubules with care; and in the two most complete expositions of the subject by Felix, the one in Hertwig's "Handbuch der Entwicklungsgeschichte," the other in Keibel and Mall's "Human Embryology," only a short summary of Mihálkovics' observations are given. But this matter is perhaps of greater pathological than embryological interest, for these phenomena resemble very closely the changes in certain metanephric diseases. Nevertheless, they occur without bacterial or outside toxic invasion, but as a part of a *normal* developmental process. They deserve, therefore, special recognition and study by the pathologist.

The degeneration of the mesonephros commences early in mammals. In the human embryo, according to Felix, even before the mesonephric development has been caudally completed, in an embryo of 5.3 mm., it involves primarily the most cranial tubules and proceeds rapidly to embryos of 21 mm. This constitutes the first and essential part of mesonephric degeneration. The second period is said by Felix to commence very gradually, progresses slowly and is characterized by incorporation of the middle mesonephric portion into the genital system. All other parts undergo complete retrogression. It will be remembered that of the remaining parts in the female (paroophoron, duct of Gärtner, parovarium), none retains any visible function, while in the male (vasa efferentia, coni vasculosi, paradidymis, epididymis, vas deferens, ejaculatory duct, seminal vesicles), the mesonephric remnants become new functionating structures.

A study of the degeneration of the mesonephros involves necessarily the consideration of three questions:

1. What are the histological changes leading to annihilation of the organ?

2. What is their cause?

3. What is their significance?

1. It has already been noted that the most complete study of the histological changes leading to loss of the mesonephros is that of Mihálkovics made in 1885. This description is so excellent and characteristic that I shall quote it in full. Mihálkovics studied the lesion in chicks,

commencing with the eighth to ninth day of incubation, and in rabbits of from 16 to 18 mm. lengths: at these times the formation of mesonephric buds and tubules has practically come to a standstill, the tubules no longer develop convolutions, but remain stationary, while the large venous sinuses recede and assume the dimensions of usual blood-vessels. The interstices and recesses thus formed are filled by connective tissue which previously has existed only sparingly and loosely between the tubules. At the same time the epithelium of the convoluted tubules gradually loses the character of secretory cells and assumes the shape of ordinary cylindrical cells with a greater affinity for stains than possessed before. The tubules persist in this state, in chicks, from the eighth to the sixteenth or seventeenth day, in the rabbit to a length of from 3.5 to 4 cm. Then they commence to obliterate. The first evidence of this obliteration is a narrowing of the convoluted tubules with a progressing small-drop fatty degeneration of the lining cells. Ultimately the cell bodies undergo a fatty dissolution and fill the lumen of the tubules. Instead of canals there remain thus solid cell cords which consist of still recognizable tubular cells. These are more or less concealed by the richly cellular stroma and may, therefore, escape attention. In the meantime the malpighian corpuscles have become involved in the process. Their loss commences with obliteration of the afferent vessels, and the atrophic glomerulus no longer fills the capsule, but leaves a space between the tuft and capsule wall. Finally, the capsule, following the receding glomerulus, retracts and becomes attached to the tuft. In sections these remnants of malpighian corpuscles resemble cell buds or groups lying between obliterating mesonephric tubules. One other point was fully noticed by Mihálkovics, although he drew from it no theoretical or hypothetical conclusions, namely, that as the mesonephros shrinks, the medially attached genital gland enlarges, thus reversing the relative size of the two organs: "Primarily the small genital gland rested on the medial side of the massive mesonephros; after its contraction insignificant rests of the mesonephros lie on the lateral side of the now huge genital gland." Mihálkovics, however, did not trace further the details of this impressive phenomenon. Later investigations have generally recognized and recorded it also without further comment.

The recent observations of Winiwarter and Sainmont on the organogenesis of the cat's ovary have supplemented in certain details Mihálkovics' work. They found that the transformation of those mesonephric parts which go to form epoophoron and paroophoron commences with a pigmentary degeneration of the lining cells of the convoluted tubules. This epithelium becomes swollen, coarsely granular and later desquamated, and in advanced degeneration narrow tubular canals are found whose lumen is filled by pigment-granules. It will be observed that the

changes described by Mihálkovics and Winiwarter and Sainmont bear close resemblance to pathological lesions. A further study, especially regarding their histogenesis and causes, seemed, therefore, desirable, for such knowledge might prove of value in the interpretation of pathological processes.

The material at my disposal was by force of circumstances limited, so that observations could not be made on a desirable extensive and detailed scale. Much remains hidden which must be cleared by future investigation. But the results are perhaps of sufficient interest to warrant this fragmentary report, more especially as they differ somewhat from previous views.[8] The available material consisted of rabbit embryos from the fourteenth to the twenty-sixth day of intra-uterine life, and marsupial embryos of *Dasyurus viverrenus*, beginning with the fifth to sixth day and extending to the nineteenth day. To obtain the full age of these embryos, about eight days intra-uterine life must be added.

These periods comprise from the early to the most active stages of mesonephric destruction. The lesions appeared essentially the same in both animals. The results are, therefore, presented together.

Certain histological modifications distinguish the destruction of the anterior portion of the mesonephros, which enters the service of the sexual gland from the posterior one, which is completely lost. The involvement of both, however, becomes evident immediately after beginning growth of the sex gland and goes hand in hand with the latter's development and differentiation. In rabbits this commences from the fourteenth or the fifteenth day, in the dasyurus after about nine days of marsupial life, and then proceeds with great rapidity. To begin with the changes in the anterior portion of the mesonephros which lies in closest contact with the sex gland: when an enlargement of the sex gland, which until then has only formed a thickening or tubercle on the medial mesonephric side, is noticeable, there occurs in the vicinity of attachment to the mesorchium or mesovarium a small cell proliferation which leads by rapid extension to a marked interstitial cell infiltration of the neighboring parts. It thus involves diffusely the periglomerular tissue, but also spreads peripherally under the outer capsule. From these points it penetrates by streaks into the glandular parts between the convoluted tubules.

The periglomerular infiltration surrounds and compresses the malpighian corpuscles, and involving Bowman's capsule, invades their interior and gradually obliterates it. The replacing cells have all the appearances of connective-tissue cells; first round, they soon acquire a distinct small and large spindle shape. During this process the glomerular tuft is much

8. I intend to publish at some later day a full, illustrated account of mesonephric degeneration.

engorged, and hemorrhagic extravasations into the capsule, sometimes sufficient to destroy the whole tuft, may occur. This loss of malpighian corpuscles in the anterior portion appears to be one of the earliest and most rapid manifestations of mesonephric degeneration.

Proceeding from this focus, streaks of interstitial cells extend to the territory of the tubules, where they meet similar invading cells from the subcapsular tissue. This progress leads to marked thickening of the previously very thin intertubular septa of the convoluted tubules, and is associated with a rather irregular engorgement and dilatation of capillaries.

Coincident and probably not, or at least not solely, dependent on this cell infiltration, is marked parenchymatous degeneration of the cells lining the tubules, closely followed by desquamation and proliferation of this epithelium. The cells become succulent, swollen, coarsely granular, in many pigmentary granules appear, or small to large vacuoles (fat?) and hyaline drops. They are then desquamated as a whole, or discharge granular masses and pigmented granules into the lumen, forming casts. The nucleus is well preserved to the last, and seems to succumb by karyolysis.

Proliferation of the epithelium is very active, so that frequently several cell layers are formed. But the new cells are soon overtaken by a similar fate. In tubules in which the cells appear more resistant or are perhaps later attacked, they seem to become higher and narrower, cylindrical, as Mihálkovics terms it, before they also undergo disintegration.

Ultimately, the tubules are invaded and compressed by interstitial cells from the septa, broken and obliterated. The collecting, straight tubules remain well preserved until late and are apparently lost by cell overgrowth without exhibiting the degenerative changes so conspicuous in the convoluted tubules.

Thus the anterior part of the mesonephros is generally overgrown by cellular fibrous tissue and contracts, and the remaining uninvolved tubules of the middle portion are drawn toward the territory of the meanwhile enormously enlarged sex gland, where they may be recognized for some time surrounded by richly cellular connective tissue.

Somewhat different seems to be the fate of the posterior portion of the mesonephros which is doomed to complete annihilation. Degenerative features seem to predominate in it almost entirely, and sclerotic changes do not take an active part in its destruction.

The changes appear very nearly about the same time as anteriorly, possibly somewhat later. Toward the end they proceed with great speed.

The glomerular coils become cellular and show swelling, turbidity and gradual fusion of capillary loops. This may involve at a time only a number of loops, and the remaining ones show then a marked capillary dilatation and engorgement. The fused coils become generally adherent

to some part of the capsule, very much as in nephritis, the glomerulus retracts and the capsular space is widened. The capsule wall shows usually swelling, but no cellular thickening, and remains intact until the whole capsule collapses from loss of the glomerulus. Such pictures resemble those described by Mihálkovics, but I have been unable to observe the swelling and obliteration of the glomerular afferent vessel to which he ascribes glomerular retraction and loss. On the other hand, the degenerative changes in the convoluted tubules are similar to those in the anterior portion, and there exists also irregular intertubular engorgement and dilatation of capillaries.

Cell overgrowth, which forms so conspicuous a feature of the destruction of the anterior part, is posteriorly absent in the beginning. Only later, in advanced degeneration, foci of spindle-cells are seen between the tubules, around capillaries, and somewhat more abundantly at the periphery under the capsule. It is not possible to state how far they may enter into the very last phases of destruction. These are very difficult to determine, as they proceed with extraordinary rapidity. In rabbits the whole disappearance is carried on from the nineteenth to the twenty-sixth day, and the final solution of the posterior part between the twenty-first to the twenty-fifth day. On the twenty-fifth day very little remains.

During the early stages of the lesion, new tubules are said to be formed. Felix attributes these either to scattered cells in the surrounding mesoderm or to budding of tubules and malpighian corpuscles. With regard to this it may be stated that no evidences of budding of either tubules or malpighian corpuscles were seen in this series, although cell proliferation within tubules and glomeruli occurs.

The lesions briefly described above attract attention on account of their similarity in character, genesis and distribution to certain types of degenerative and productive nephritis. Indeed, so close is this relation that one may speak of an acute destructive mesonephritis.

An investigation into its cause and significance seems, therefore, worth while.

2. Three possibilities may be entertained in regard to the cause of mesonephric retrogression:

A. It may be due to crowding of the tubules within a small space. To this has been attributed the early involvement of the most cranially situated tubules. Felix, however, regards this as a very subordinate, if any, cause for the reason that the mesonephric arteries show at no time any indication of crowding by being bent in their course, but, on the contrary, remain perfectly horizontal. There is, moreover, as seems to me, no histological evidence of crowding at any time, nor is the histogenetic picture of the retrogression itself in harmony with this view.

B. Mesonephric loss may be essentially due to interference and obliteration of its blood-supply. This is also not borne out by the facts.

In the first place, the degeneration commences at a time when the mesonephric blood-supply is still quite adequate and sufficiently intact to allow the formation of new tubules. In the second and most important place, the histological picture corresponds in no way to what is to be expected after quantitative interference with the blood-supply of an organ. Two things may occur as the result of this, atrophy or infarction. Here we have neither, but an extensive active cellular proliferation and invasion associated with a characteristically located rapid parenchymatous degeneration. Both go far beyond the limits of the results of simple quantitative interference with the vascular supply of an organ. Furthermore, the irregular streaky advance of the lesion, attacking certain parts while others are still intact, the capillary engorgement, degenerative cell waste and parenchymatous and interstitial cell proliferation leading to extensive cicatrization relate it to the inflammatory conditions.

C. There remains then the possibility of an influence which is conveyed to the mesonephros from other parts. In this connection attention is immediately arrested by the peculiar relation in which growth and development of the medially attached sex gland stand to mesonephric destruction.

They commence in closest association with each other and progress in inverse ratio. Indeed, rapidity of growth and differentiation in one, and corresponding destruction and loss of the other are impressively associated. In particular, the earliest and most pronounced changes leading to loss of malphigian corpuscles in the anterior part take place at the point of most intimate connection, and the degeneration appears to acquire momentum with progressing maturity of the genital gland. We are confronted, therefore, by the question of a possible antagonistic influence of the latter on the mesonephros, and it is necessary to inquire how well such an idea is founded.

3. The idea of antagonism between cells, tissues and organs is by no means new. It has been emphasized by several biologists that among the forces which shape phylogenetic and ontogenetic evolution, antagonism between organs may be of as great an importance as correlation or altruism. As early as 1876 Boll, in "Princip des Wachsthums," said:

> Microscopic investigation has demonstrated in the peaceful egg the existence of highly dramatic processes which, out of compliment to a much-beloved terminology of doubtful value, I shall link together under the title of "the struggle of the tissues."[9]

9. Die mikroskopische Untersüchung hat im Frieden des Eies die Existenz höchst dramatischer Vorgänge nachgewiesen, welche ich aus Konzession an eine sehr beliebte Terminologie von zweifelhaften Werthe unter der Bezeichnung des *Kampfes der Gewebe* züsammenfassen will.

It is well known that Roux has elaborated these ideas in his well-known monograph, "Der Kampf der Theile im Organismus," and recently von Hansemann, in "Deszendenz und Pathologie," has added another illuminating discussion of the subject. The individual organism reflects to a great extent the forces which move a community of individuals. In phylogeny, correlation and altruism of individuals are supplemented by a necessary antagonism to insure preservation and progress of the individual within the community; in ontogeny similar forces are repeated in the relation of cell to cell, tissue to tissue, organ to organ. Thus weak and unnecessary organs which can no longer hold their own in the community of organs are removed, and the organism is freed from what would otherwise be a disadvantageous burden in its evolution. We have certain reasons to suspect that on such antagonistic actions rest the disappearance of various embryonic structures, the thymus, for example; but in most instances this suspicion can only be vague. The case under discussion, however, presents apparently a definite instance of growth and development of one organ at the expense of another. How is this accomplished?

Goethe, St. Hilaire and Roux have drawn attention to the fact that nutritive struggle plays an important rôle in mutual antagonism, for if one part receives for its growth a large supply of the available nutriment, others must suffer. They termed this the law of compensatory growth. Darwin, however, while admitting its importance, rather regarded it as a subordinate influence. Roux believes, nevertheless, that such nutritional influences are by no means always of the ordinary direct mechanical circulatory nature, but of a much more complicated character. He draws attention to the fact that women who nurse for years are very susceptible to osteomalacia because, he believes, of constant withdrawal of calcium salts in the milk, which is, therefore, unavailable for the bone. The antagonism or, as he calls it, "struggle of the mammary glands with the bones," is characterized by greater strength of the former to attract and hold calcium salts. Later investigators have endeavored to trace this to a direct influence of the sex gland. It is possible that a nutritive struggle may enter into the antagonistic relations of mesonephros and sex gland. But it must be considered doubtful whether it can be regarded as the sole cause of mesonephric loss, for certain features distinguish this from nutritive disturbances. The mesonephros is apparently attacked by a distinctly irritative, destructive and, if the term is permissible, formative stimulus which leads to its speedy exitus.

Again, in modern investigations many phenomena of organic interrelation are attributed to hormones. Hormones may be defined as messengers from one organ to another which either excite or inhibit

functional activities. The process under consideration can, therefore, hardly be grouped within the ordinary understanding of hormone action. Its exact nature remains unknown.

Even if we were obliged to leave this point in abeyance, however, we may go further and ask whether the possibility of similar antagonism between organs may not outlast embryonic life and continue throughout postnatal existence. Here we touch on points of great importance to the pathologist. We are dealing in the embryo, as has been shown, with a mesonephritis which cannot be attributed to bacterial or an outside toxic influence. It is the result of a true auto-intoxication. We may, therefore, ask more specifically, whether similar phenomena continue after birth. In other words, if, as seems highly probable, antagonism of organic units is one of the moving forces in the life of all individuals from practically the first division of the ovum to death, may it not enter more than hitherto supposed into production, manifestation and outcome of diseases? Perhaps etiology has for practical reasons been too anxious to attach an almost exclusive importance to external causes of disease, and has neglected a study of those internal conditions which prepare and make possible the development and specificity of morbid processes.

The consideration of these problems reaches at present the borderland of pure speculation; but future investigations may put them, like many other at first sight phantastic views, on a sound disputable basis.

Finally, we must record what is known of any possible function of the mesonephros in animals in which the renal activity has been handed over to the metanephros.

Has the persistence of the mesonephros through at least part of the evolution of the individual any significance? This problem is intimately connected with the previous one.

Johannes Müller, who discovered its capillary tufts, believed that the wolffian tubules secreted urine which passed through the wolffian duct into the cloaca or allantois.

Mihálkovics came to the conclusion that the amniotic metanephros does not, in all probability, assume the functions of urine secretion suddenly or abruptly, as a considerable part of the wolffian body still exists at a time of advanced development of the metanephros. There exists, in his opinion, a period when both organs functionate jointly as excretory organs. Mihálkovics sought support of this contention in the phylogenetic history of the mesonephros, particularly in its continuation through the early part of postnatal life in reptiles. This, in Mihálkovics' opinion, would indicate that among the lowest forms of amniotes the metanephros is assisted in some way and at least for a time by the mesonephros. He concludes that in the evolution of amniotes there

existed antecedents, now extinct, in which the mesonephros persisted throughout life, while the metanephros remained incomplete and small. This first anlage of the metanephros gradually developed through increasing and complicated demands to the size and importance of the present organ.

Bonnet[2] also states that he has found urine in the mesonephric tubules of pig embryos commencing with 1 cm. length and in the metanephric tubules in embryos from 8 cm. length.

In man the mesonephros acquires, as already emphasized, a considerable high state of differentiation. There are, nevertheless, certain points which speak against its excretory activity in man. Most important is in this respect its early degeneration, which, according to Felix,[2] commences even before the organ has fully developed. Again, man lacks a vesicular allantois and urinary bladder in early life, and the urogenital sinus does not open to the outside until the embryo has reached a length of 14 mm. (Bonnet). Bonnet states that the excretion of nitrogenous waste-products and elimination of water probably take place in the region of the allantois or by the chorionic circulation, and later by the umbilical vessels in the placenta. It is, therefore, generally held that the mesonephros in mammals and particularly man possesses no excretory renal function.

Is then the mesonephros a purely rudimentary organ which enters in no way into the welfare of the organism? Its very existence and high grade of differentiation would argue against it, for complete loss of function leads usually to either entire loss or a very incomplete appearance of an organ in later stages of phylogenetic evolution. This, for example, is the case with the pronephros, whose very incomplete appearance in higher amniotes excludes almost with certainty the possibility of any function whatever.

Following and guided by the history of some other organs of internal secretion, an effort has lately been made to find similar evidences for the mesonephros; but practically very little has been accomplished in this regard.

The recent observations of Kermauner[10] offer perhaps a clue in this direction. According to him the absence of the wolffian bodies is incompatible with life and leads to early death of the fetus. He, therefore, regards the mesonephros of higher animals no longer as an organ of excretion, but of internal secretion (hormones) and assimilation. Kermauner points in support of this idea to the rich blood-supply of the

10. Kermauner: Das Fehlen beider Keimdrüsen, Beitr. z. path. Anat., von Ziegler (now Marchand and Aschoff), 1912, liv, No. 3, p. 478.
Wolff, B.: Ueber foetale Hormone, in Handbuch der Biochemie von Carl Oppenheim, Ergänzungsband, 1913.

mesonephros with the numerous segmental arteries and veins which go to form its characteristic portal circulation.

He concludes that the wolffian body is essential for certain periods of life, first in the development of the placenta, and secondly in complementing assimilative functions of other fetal parts until the appearance of permanent organs, notably the liver, renders this unnecessary. The specific functions of the wolffian body are then no longer required, the fetus passes to new phases of life, and the mesonephros makes its exit.

It must, however, be mentioned in this connection that the anlage of the mesonephric portal circulation in man is, according to Felix, only very short-lived, since in his observations the subcardinal veins and the transverse mesonephric branches are in act of degeneration in an embryo of 12.5 mm. greatest length. Kermauner's views are in their attempt of direct definition of mesonephric functions at the best very hypothetical, and need additional facts for sound basis. On the other hand, it is of course well known that certain organs of internal secretion or specific metabolic activity[11] functionated originally as organs of external secretion. They present, therefore, the peculiar phenomenon of functional mutation during phylogenetic evolution with morphological persistence. Perhaps the thyroid gland furnishes the best example of this type, for whether we agree with Gaskell's extreme view that these glands were originally uterine, or whether we regard them, more conservatively, as derivatives of mucous glands,[12] the fact remains that they have undergone a functional mutation but have retained the acinar tubular structure which is characteristic of glands with an external duct.

Two possibilities may exist in this transformation of glands of external secretion to glands of internal secretion and specific metabolic functions. First, the glands possessed originally only the function of external secretion, and the others have been acquired or developed entirely *de novo* after the usefulness of the gland as an organ of external secretion has been ended.

This would seem an unsatisfactory and unintelligible explanation, for it accounts in no way for the manner in which the new functions originated while structure and arrangement of the parts continued. On the contrary, one would expect here as in other tissues that loss of function would go hand in hand with loss of structure or alteration in

11. I use the two terms "internal secretion" and "specific metabolic activity" because the influence of these glands may be due to other factors besides internal secretion. Certain observations by Milne and Peters (Observations of the Glycolytic Power of the Blood and Tissues in Normal and Diabetic Conditions, Jour. Med. Research, 1912, xxvi, 415) make this probable for the pancreas.

12. Gaskell: See Footnote 2. For the evolution of the ductless glands consult Hertwig's Handbuch der Entwicklungsgeschichte.

function with alterations in structure and rearrangement of parts. This is the rule under physiological and pathological conditions.

A second possibility seems, therefore, much more plausible and stands on some facts: glandular organs possess from their origin functions of external and internal secretion and specific metabolic activities. The exercise of these is initiated, developed and modified by the environment. If thus, through evolutionary agencies, the necessity or even possibility for external secretion no longer exists, it ceases, but the gland still retains its functions of internal secretion and specific metabolism. If now, further, no soil for this internal secretion has been developed,[13] or if the activity as a special metabolizer is no longer required, the gland is lost from disuse. It is very possible that under such circumstances its removal is expedited by the antagonistic action of other glands. If, however, a receptive soil for its internal secretion has been created and is retained during consecutive periods, or if its specific metabolizing function is adapted for succeeding evolutionary stages, the gland is not only retained, but strongly specializes under these stimuli in either one or both of these directions. It may thus acquire an even greater influence on the internal economy of the organism than it formerly possessed with divided functions and, in turn, may play an important part in future evolution.

We know, for example, that man still possesses several glands with external secretion which in addition are capable of and do exert strong internal influences, notably the testicle and the pancreas. The older idea that these organs contained specific structures for internal secretion or special metabolic purposes has been replaced by the probably more correct view that the whole of the gland's parenchyma is potent in these regards. This is certainly true of the pancreas,[14] probably also of the testicle, for while an internal secretion has been attributed to its interstitial cells, it seems certain that the glandular secretion which, moreover, is discharged only at relatively long intervals, exerts a very decided influence particularly on muscles and brain.

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13. In discussions of the disorders of internal secretion, perhaps insufficient attention has been paid to the importance which the soil may exercise in this connection. It is evident, however, that the effect of an internal secretion depends not only on the function of the gland, but also on the condition of the particular soil which has become receptive to the secretion. It would seem, therefore, that disturbances may arise not only through glandular disorders, but also through developmental or acquired faults of the soil. This mutual relation may play an even greater part when close anatomical and physiological connections existed during embryonic life. Finally, it seems not improbable that internal secretions may be modified on their way by passage through other glands. This opens another opportunity for variations.

14. Milne and Peters: See Footnote 10. Potter and Milne: Bronzed Diabetes, Am. Jour. Med. Sc., 1911, cxliii, 46.

The interesting and peculiar mutation of function with morphological persistence in glands is then rendered intelligible when we regard it as a relative one, representing rather a specialization than a new acquisition. The rôle which these organs are to play in life and the duration of their existence are determined by first, the period of evolution at which the mutation occurs, secondly, the nature of their internal secretion or metabolism, and thirdly, their relationship to a particular soil or phase of general metabolism. Consequently these organs become arranged according to periods of evolution, and appear and disappear with them. Here as elsewhere ontogeny appears as a recapitulation of phylogeny.

The mesonephros, although short-lived, may, nevertheless, exercise an influence on human fetal life and development not unlike that of other fetal and infantile structures. It cannot, in view of what has been presented above, be denied. But this question is, like the others, still far from being solved, and we possess to-day only two definite observations in this regard: its complete and high degree of development and differentiation, and the statement advanced by Kermauner that its absence is not compatible with life. A third, but much more uncertain, point might be construed from its relation to the genital gland. This appears to owe its inception, growth and differentiation to the mesonephros, which it ultimately consumes. Finally, a fourth, but equally uncertain conclusion, might be drawn from the histological pictures observed by Mihálkovics and Winiwarter and Sainmont. These may indicate a secretory activity. But we know from observations of the adult metanephros that such pictures are misleading[15] and not conclusive.

These, then, are some of the problems which confront us in the study of renal evolution.[16] That their bearing is not only of special, but also of very general pathological interest, seems not doubtful. I enter these pages as a plea for their investigation and for a more careful consideration of ontogeny and phylogeny by pathologists.

I have in conclusion many grateful acknowledgements to make: Prof. Arthur Keith, F. R. S., director of the Hunterian Museum at the Royal College of Surgeons, London, allowed me study of his specimens and put his technical assistant at my disposal. Prof. James P. Hill, F. R. S., of University College, London, gave me free use of his collection of *Dasyurus viverrenus* and much advice. To Prof. Richard Assheton, F. R. S., of the University of Cambridge I am particulariy indebted, not only for many carefully selected sections of rabbit embryos, but also for a great deal of active help and counsel. Last, not least, to my colleagues in the Gordon laboratory, Guy's Hospital, Drs. Laidlaw, Goodhart and Chisholm, I owe material and constant kind and helpful interest.

29 St. James' Street, S. W.

15. Aschoff, L.: Zur Morphologie der Nierensekretion, Verhandl. d. deutsch. path. Gesellsch., Jena, G. Fischer, 1912.

16. I have not touched at all in this communication on any possible differences in this process between male and female and, further, what determines the preservation and subsequent incorporation into the sexual system of certain mesonephric tubules. These problems need special investigation.

A METHOD DEVELOPED FOR OBTAINING A STANDARD WASSERMAN ANTIGEN *

CYRUS W. FIELD, M.D.
NEW YORK

This method has been developed in the Pathological Department of Bellevue Hospital during the past six months as a result of a study on the routine Wassermann reactions of five thousand patients. These patients have stayed in the hospital three days or longer; otherwise there has been no choice of subjects.

For a long time it has been felt that the only variable reagent in the Wassermann reaction has been the antigen. Antigens have been made up from the alcoholic extracts of human syphilitic livers, human hearts and ox-hearts. The values of these various antigens have differed

TABLE 1.—READINGS WITH THE SAME

Antigen	Serum A Antigen, Dil., c.c.							Serum B Antigens, c.c.							Serum C Antigens, c.c.						
	Control	1.	0.8	0.6	0.4	0.2	0.09	Control	1.	0.8	0.6	0.4	0.2	0.09	Control	1.	0.8	0.6	0.4	0.2	0.09
Antigen 1	0	3	3	3	3	2	0	0	3	3	3	3	3	2	0	3	3	3	3	0	0
Antigen 2	0	3	3	3	3	3	0	0	3	3	3	3	3	-1	0	3	3	3	3	1	0
Antigen 3	0	3	3	3	3	1	0	0	3	3	3	3	3	3	0	3	3	3	3	0	0
Antigen 4	0	3	3	3	3	2	0	0	3	3	3	3	3	2	0	3	3	3	3	3	0
Antigen 5	0	3	3	3	3	3	0	0	3	3	3	3	3	2	0	3	3	3	3	1	0
		High, 15; low, 13.							High, 18; low, 16.							High, 15; low, 12.					

* In Tables 1 and 2, 0 = complete hemolysis, 1 = marked hemolysis, 2 = slight hemolysis Twenty-three other cases were 0. All these cases were specific as to history or lesion. Forty other serums were negative.

considerably in each batch made up. This is apparently due to variations in the organs from single individuals. For this reason the extract from guinea-pig hearts has been preferred because a larger number can be used for each batch of antigen, so that the individual factor is reduced to the vanishing-point.

THE METHOD IN DETAIL

The method of conducting the Wassermann reaction in this laboratory is as follows:

Patient's Serum.—The patient's serum is poured off from the clotted blood and centrifuged. The clear supernatant serum is removed and

* Submitted for publication March 3, 1914.

inactivated at 56 C. for half an hour. A test-tube rack containing seven test-tubes is set up, and for the half quantity of the original Wassermann reaction 0.1 c.c. of the patient's serum is pipetted into each tube.

Complement.—The complement is obtained by bleeding at least three guinea-pigs and removing the serum after from twelve to eighteen hours. This serum is diluted 1 part of serum to 8 of physiological salt solution; 0.5 c.c. of this diluted complement is added to each one of the seven tubes.

Indicator.—The indicator used to detect the presence or absence of the complement is 0.5 c.c. of a 5 per cent. suspension of sheep-cells, to which has been added 0.5 c.c. of a dilution of amboceptor of such strength that each cubic centimeter contains four hemolytic units. This mixture is allowed to incubate at 37 C. for half an hour before being added to the Wassermann system. It has been found from a study of over ten thousand Wassermann reactions that the native amboceptor occurring in human blood may be disregarded if this be done.

SERUMS WITH FIVE DIFFERENT ANTIGENS*

Control	Serum D Antigens, c.c. 1.	0.8	0.6	0.4	0.2	0.09	Control	Serum E Antigens, c.c. 1.	0.8	0.6	0.4	0.2	0.09	Control	Serum F Antigens, c.c. 1.	0.8	0.6	0.4	0.2	0.09	Control	Serum G Antigens, c.c. 1.	0.8	0.6	0.4	0.2	0.09
0	3	3	3	3	0	0	0	3	2	1	0	0	0	0	3	3	3	3	3	0	0	3	0	0	0	0	0
0	3	3	3	2	0	0	0	3	3	3	0	0	0	0	3	3	3	3	3	0	0	2	0	0	0	0	0
0	3	3	3	3	0	0	0	3	3	2	0	0	0	0	3	3	3	3	2	0	0	3	1	0	0	0	0
0	3	3	3	2	0	0	0	3	3	1	0	0	0	0	3	3	3	3	3	0	0	3	0	0	0	0	0
0	3	3	3	1	0	0	0	3	3	3	0	0	0	0	3	3	3	3	1	0	0	2	1	0	0	0	0
	High, 12; low, 11.							High, 9; low, 7.							High, 15; low, 13.							High, 4; low, 2.					

and 3 = no hemolysis.

Antigen.—The antigen that is now used is an alcoholic extract of guinea-pig hearts to which has been added enough cholesterin to bring it to the half-saturation point. When the pigs are killed the heart is removed, and all blood present in the cavities is removed by massaging with a clean towel. The heart is weighed, this weight being recorded on the jar in which the heart is preserved until a sufficient amount has accumulated. In preparing this antigen at least 300 gm. of guinea-pig heart, after having been finely ground, is extracted for fourteen days at 37 C. with 3,000 c.c. of absolute alcohol, chemically pure. It is then filtered, and half the filtrate is placed in a flask and an excess amount of chemically pure cholesterin is added. It is allowed to stand in the incubator over night. The next morning the flask is removed from the

incubator and placed in a constant temperature bath at 16 C. for three hours. This is to precipitate the excessive amount of cholesterin. It is now filtered and this saturated cholesterin solution is added to an equal quantity of the filtered alcoholic extract. Twenty cubic centimeters are removed with a pipet and placed in a weighed dish and evaporated at 56 C. to dryness. It is then placed in a desiccator for twenty-four hours over sulphuric acid and then weighed. It is thus determined what the total lipoid cholesterin concentration of this alcoholic solution is. After having determined this, it is tested for its anticomplementary effect.

In all, five different lots have been made up by this method, and they have been remarkably constant in their total lipoid concentration and their anticomplementary value, and, as will be seen from Table 1, in their antigenic value.

Dilution for Use.—One cubic centimeter of the alcoholic extract is added to as many cubic centimeters of salt solution as is necessary to make the lipoids and cholesterin weight equal to 0.1 mg. per cubic centimeter. That is, if 100 c.c. of the alcoholic extract contain 0.780 gm., we add 1 c.c. of this to 77 c.c. of physiological salt solution. This is the first antigen dilution, and 1 c.c. is placed in the second tube in the rack (the first tube containing only salt solution and no antigen, this being the control). In the third tube is placed 0.8 c.c. of this antigen dilution; in the next 0.6; in the next 0.4; in the next 0.2, and in the next 0.09. This gives us a series of antigen tubes with decreasing amounts of antigen and a constant amount of serum. Enough 0.8 per cent. sodium chlorid solution is added to each tube to make the total volume 1.6 c.c. The antigen can be diluted with the sodium chlorid solution before it is added to the tube; this is perhaps easier to do. The reading is made as soon as a control set of tubes containing complement and the various strengths of antigen, and the control tube in the individual set, show complete hemolysis.

Only four degrees of hemolysis are observed in the tubes: zero, equaling complete hemolysis; 1, marked hemolysis; 2, slight hemolysis, and 3, no hemolysis. In this way, having three degrees of inhibition for each tube and six antigen tubes, we have a total possibility of eighteen units, provided complete inhibition occurs in all these tubes. Less than 0.5 per cent. of all positive cases give eighteen or more units.

Duplicate tests of patient's serum, unknown as duplicates to the person who made the readings, came out within one or two units of each other constantly. The tests on the five different batches of antigen made up with this procedure have shown no greater differences with the same serum, that is, one or two units. This is a much more constant reading than we have been able to obtain with any antigens heretofore. With

this method the antigen is practically as constant in quality as is the amboceptor or complement.

Table 1 shows a series of readings with the same serums with the five different antigens. Table 2 shows duplicate serum readings with one of the antigens.

TABLE 2.—DUPLICATE SERUM READINGS WITH ONE OF THE ANTIGENS

Serum	Control	1.	0.8	0.6	0.4	0.2	0.09	Units of Serum	Units in Sp. Fl.
J. K.	0	3	3	3	3	3	0	15	15
	0	3	3	3	3	3	0	15	
T. R.	0	3	3	3	3	2	0	14	
	0	3	3	3	3	3	0	15	
P. C.	0	3	3	3	3	3	2	17	15
	0	3	3	3	3	3	2	17	
J. L.	0	3	3	3	3	3	2	17	
	0	3	3	3	3	3	1	16	
M.	0	3	3	3	0	0	0	9	
	0	3	3	2	0	0	0	8	

CONCLUSIONS

1. The cholesterin, guinea-pig heart antigen is readily obtained and constant in its antigenic value. (This has already been suggested by Swift and Ellis and others.)

2. A series of dilutions of the antigens is obtained by weight, which has been shown to give constant results.

3. A Wassermann reaction may now be standardized so that the readings will agree very closely in far distant laboratories.

4. In the use of this antigen the patient's serum must be inactivated.

5. There must be more than two units of complement.

6. The red cells must be sensitized by the amboceptor before being added to the system.

This antigen has been criticized as being too delicate and as giving positive results in non-specific cases.

More than five thousand cases were run in duplicate, one set with this antigen and the other with an acetone-insoluble antigen from ox-heart. There were 8 per cent. positive with the cholesterin guinea-pig heart which were negative with the ox-heart, but in none of them was it possible to exclude syphilis; in fact, the vast majority of them showed luetic infection or the results of such an infection, or had a positive history.

A more detailed paper will be published later, giving the results of this method as applied to treatment and in the individual variations of the Wassermann reaction in the same individual over various periods.

Pathological Laboratory, Bellevue Hospital, Twenty-Ninth Street and First Avenue.

A STUDY OF THE ACTION OF ATROPIN ON THE EOSINOPHIL CELLS OF THE BLOOD*

W. W. HERRICK, M.D.
NEW YORK

Our knowledge of the functions of the eosinophil cells is slight. The most important and perhaps the only well-demonstrated function of these cells is their association with the reaction of the body to the invasion of alien protein. The notable rise in number of eosinophils in the circulating blood following the parenteral introduction of various proteins is now well known. By the work of Schlecht,[1] Schlecht and Schwenker,[2] Schittenhelm, Weichardt and Grisshammer,[3] Stäubli,[4] Herrick,[5] and Ahl and Schittenhelm[6] it has been thoroughly established that the subcutaneous, intraperitoneal or intravenous introduction of alien proteins at longer or shorter intervals is followed by a definite eosinophilia of the blood. Particularly is this a feature of the post-anaphylactic stage following the injection of protein at intervals of five days or more. The eosinophilia following administration of diphtheria antitoxin and of tuberculin has been recognized for a still longer time. Possibly the eosinophilia observed during convalescence from various infections is a manifestation of the same response on the part of the living organism to the invasion of foreign protein.

Since the problem of immunity is bound up with that of the adaptation of the body to alien protein material, and since the eosinophils have such close relation to the process, any study throwing light on the

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* From the Department of Practice of Medicine, College of Physicians and Surgeons, Columbia University, New York.

1. Schlecht: Ueber die Einwirkung von Seruminjectionen auf die Eosinophilen und Mastzellen des menschlichen und tierischen Blut, Deutsch. Arch. f. klin. Med., 1910, xcviii, 308.

2. Schlecht and Schwenker: Ueber die Beziehungen der Eosinophilie zur Anaphylaxie, Deutsch. Arch. f. klin. Med., 1912, cviii, 405.

3. Schittenhelm, Weichardt and Grisshammer: Eiweissumsatz und Ueberempfindlichkeit, Ztschr. f. exper. Path. u. Therap., 1912, x, 3, 412; Ueber den Einfluss parenteral verabreichter Proteinsubstanzen verschiedener Herkunft auf das Blutbild, ibid.

4. Stäubli: Die klinische Bedeutung der Eosinophilie, Ergebn. d. inn. Med. u. Kinder., 1910, vi, 12; Ueber Eosinophilie, Samml. klin. Vortr., 1909, clxvii, 43.

5. Herrick, W. W.: Experimental Eosinophilia with an Extract of an Animal Parasite: Its Relation to Anaphylaxis and Certain Clinical Problems, THE ARCHIVES INT. MED., 1913, xi, 165.

6. Ahl and Schittenhelm: Ueber experimentelle Eosinophilie nach parenteraler Zufer verschiedener Eiweissstoffe, Ztschr. f. d. ges. exper. Med., 1913, i, 111.

functions of these important cells offers at least the chance of illuminating the larger problem. It has been with this possibility in mind that the study of the effect of atropin on the eosinophils was undertaken.

Ehrlich, cited by Mayer,[7] has remarked that too little experimentation has been done on the relation of the eosinophils to chemical substances, and that the results thus far obtained have been inconstant because insufficient material has been used in such experimentation. In the present study of the influence of a chemical substance on the eosinophils it is thought that sufficient material has been used to meet this very appropriate objection.

Atropin was not selected at random; neither is the problem the narrow one that would at first thought appear. Auer[8] has recorded the observation that anaphylactic shock in guinea-pigs may be prevented by the injection of from 0.5 to 1 mg. of atropin. Since eosinophilia so constantly follows recovery from anaphylaxis, it is of interest to learn whether or not atropin influences the cellular reaction. Again, a clinical problem is concerned in the question of the control of the eosinophils by atropin. The term "eosinophil diathesis" has found its way into medical terminology. Under this heading are classified those persons prone to bronchial asthma, mucous colitis, urticarias and allied conditions that have as a frequent accompaniment an eosinophilia of the blood. Eppinger,[9] in a description of the somewhat vague clinical concept called "vagotonie," states that cases characterized by excessive functioning of the autonomic nervous system, "vagotonikers," show an excess of eosinophils in the circulating blood, which excess can be reduced by atropin. No experiments are cited in support of this statement. It has been suggested elsewhere that there is a relation between the problems of anaphylaxis, eosinophilia and that of bronchial asthma; that substances capable of influencing the eosinophil cell might be of clinical use in dealing with that baffling symptom asthma. With these questions in their somewhat complex relations in view, the study of the influence of atropin on the eosinophils was undertaken.

METHODS OF STUDY

Guinea-pigs were chosen for experiment for reasons sufficiently explained in numerous other communications by various writers.[10] The

7. Meyer: Die klinische Bedeutung der Eosinophilie, Berlin, S. Karger, 1905.

8. Auer: The Effect of Vagus Section on Anaphylaxis in Guinea-Pigs, Jour. Exper. Med., 1910, xii, 638.

9. Eppinger and Hess: Die Vagotonie, Samml. klin. Abhandl., 1910, Nos. 9 and 10, p. 41. Hopkins: A Clinical Study of Vagotonia, THE ARCHIVES INT. MED., 1913, xii, 556.

10. For discussion of the blood-picture and blood reactions of the guinea-pig the following may be consulted:

Opie: The Occurrence of Cells with Eosinophil Granulations, and Their Relation to Nutrition, Am. Jour. Med. Sc., 1904, cxxvii, 217; An Experi-

blood-counts were made by the method most commonly in use in clinical work. Blood was taken from the ear. The usual pipet giving a dilution of 1 : 10 was used to determine the total number of leukocytes, while for the differential count smears were made, stained by Wright's stain, and 200 cells counted with the oil-immersion lens. Several control counts were made before beginning each experiment. In all ten guinea-pigs were used. Atropin was given in a 10 per cent. solution by hypodermic injection. The horse-serum was given intraperitoneally. The dates of the blood-counts, and the amounts and times of administration of atropin and of serum are detailed in the accompanying charts, together with the curve illustrating the variations in total numbers of the eosinophil cells in a cubic millimeter of blood. The dosage of atropin was large, allowing for the tolerance to this drug shown by the graminivora.

PROTOCOLS OF EXPERIMENTS

Experiment 1.—Guinea-Pig 1 weighed 640 gm. Control counts showed constant eosinophilia, from 817 to 4,867 in a cubic millimeter. From June 27 to July 23 eleven doses of atropin sulphate, 0.002 gm., were given. From July 29 to August 13 ascending doses of from 0.04 to 0.15 gm. were given. Exact amounts

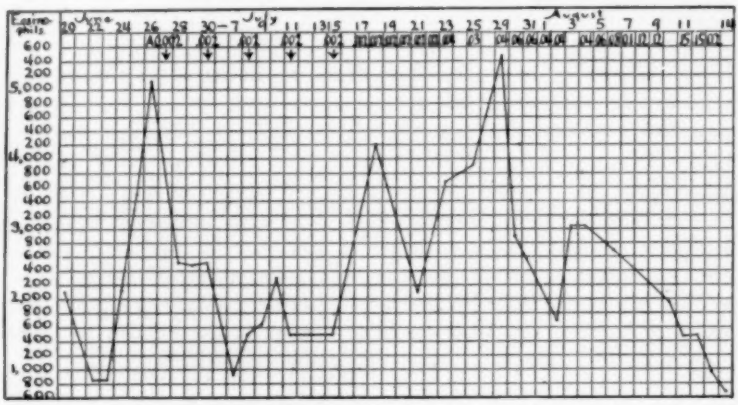

Chart 1.—Experiments on Guinea-Pig 1. In the charts the atropin dosage is given in grams, the figures preceded by the letter "A," and the serum is given in cubic centimeters, the figures preceded by the letter "S."

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mental Study of the Relation of Cells with Eosinophil Granulation to Infection with an Animal Parasite, ibid., 1904, cxxvii, 477.

Staubli: Trichinosis, 1909, p. 107 et seq.

Gulland and Goodall: The Blood, 1912, p. 76.

Kanthack and Hardy: Wandering Cells of Mammalia, Jour., Physiol., London, 1894, xvii, 81.

Herrick, W. W.: Footnote 5.

Klineberger and Karl: Die Blutmorphologie der Laboratoriumstiere, Leipsic, 1912.

and times of administration may be learned from Chart 1. There was definite fall in the eosinophil curve during the last ten days of the experiment, the period of administration of huge doses of atropin. At the same time there were toxic symptoms: loss of weight, roughening of the coat, weakness, loss of appetite, lessened activity, etc. Weight August 14, 592 gm.

EXPERIMENT 2.—Guinea-Pig 2 weighed 670 gm. Control counts showed eosinophils varying in numbers from 1,950 to 5,340. From June 30 to July 22, ten doses of atropin sulphate 0.002 gm., were given on alternate days without influence on the numbers of these cells. From July 23 to August 12, atropin sulphate in amounts from 0.04 to 0.15 gm. were given daily or every second day. No notable fall in the eosinophil curve was apparent. Toxic symptoms were

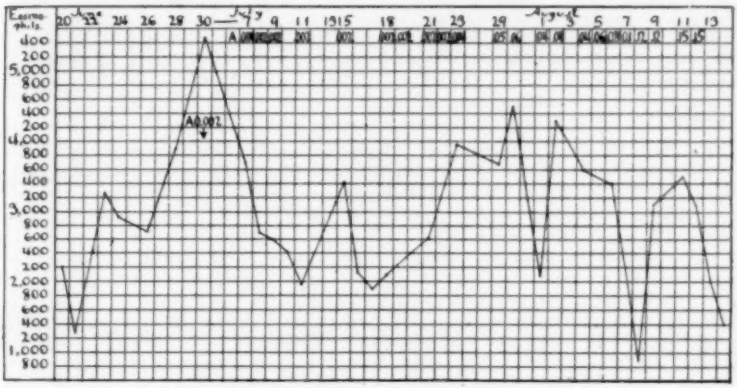

Chart 2.—Experiments on Guinea-Pig 2.

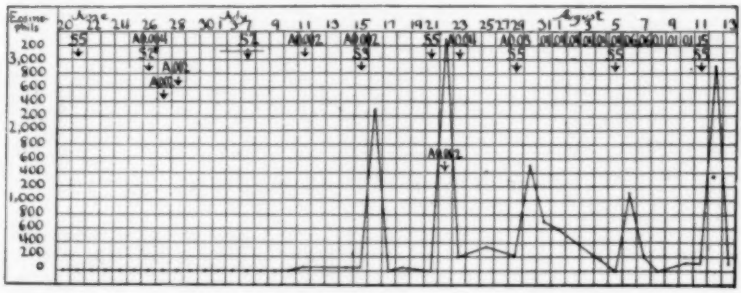

Chart 3.—Experiments on Guinea-Pig 3. * June 26, the serum was given one hour after the atropin.

produced of a character similar to those described in the case of Guinea-Pig 1. Weight August 13, 618 gm.

EXPERIMENT 3.—Guinea-Pig 3 weighed 500 gm. During the control period no eosinophils were found in the circulating blood. Interval injections of serum after the third were followed by the usual eosinophilia. Atropin in large amounts, from 0.03 to 0.15 gm. on the day before that of the injection of serum apparently inhibited the rise in number of eosinophils in two instances, but in one instance

did not prevent the development of a marked eosinophilia, which occurred despite daily injections of large amounts of atropin for a period of twelve days. The results in this instance are therefore contradictory.

EXPERIMENT 4.—Guinea-Pig 4 weighed 480 gm. The eosinophil response on the part of this animal to repeated injections of serum was slight though definitely present. This may have been due to the apparent general feebleness of the animal, which succumbed to a dose of atropin sulphate, 0.1 gm. Atropin given in doses of 0.002 or of .02 gm. at the time of administration of serum had no apparent effect on the subsequent rise in number of eosinophils.

EXPERIMENT 5.—Guinea-Pig 5 weighed 500 gm. This animal was used as a control to show the effect of interval injections of serum on the eosinophils. Atropin sulphate, 0.04 gm., given at the time of the sixth inoculation of serum, apparently exerted an inhibitory influence on the subsequent eosinophilia. This eosinophilia, though present, was much less than in the two preceding instances following inoculation of serum alone, and may possibly have been a result of the action of atropin.

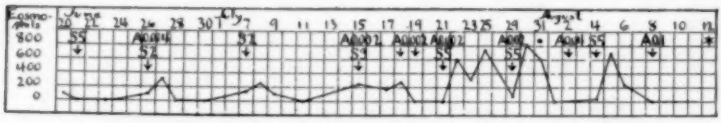

Chart 4.—Experiments on Guinea-Pig 4. * Ill, weak, died.

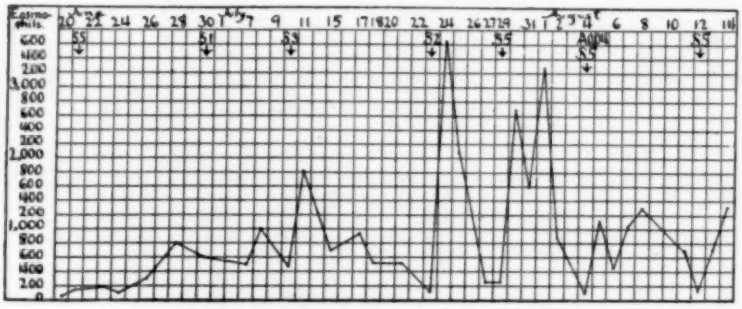

Chart 5.—Experiments on Guinea-Pig 5.

EXPERIMENT 6.—Guinea-Pig 6 weighed 480 gm. Control counts of eosinophils showed from 270 to 648. Three injections of serum alone at intervals of from seven to twelve days were followed in case of the last two by increase of eosinophils, the maximum number being 4,638. July 29, atropin sulphate, 0.02 gm., with serum, 5 c.c., was injected. There was response on the part of the eosinophils, their numbers rising from 836 to 2,362 forty-eight hours later. August 4, atropin, 0.04 gm., was injected. August 5, serum, 5 c.c., was injected. Eosinophils, absent on the 5th, rose to 1,120 the 6th, a much lower figure than that seen after other injections of serum alone in this animal. August 11, eosinophils were 576. Atropin, 0.1 gm. was injected. August 12, eosinophils were 1,581. Serum, 5 c.c. was injected. August 14, eosinophils were 864. In this instance the usual rise in the number of eosinophils was lacking.

EXPERIMENT 7.—Guinea-Pig 7 weighed 500 gm. In this animal enormous and finally lethal doses of atropin did not prevent the development of the eosinophilia

consequent on the interval injections of serum, such an eosinophilia being present on the day of death.

EXPERIMENT 8.—Guinea-Pig 8 weighed 560 gm. Control counts showed a moderately high eosinophilia, averaging 1,300 in a cubic millimeter. Atropin sulphate, 0.05 gm., was given twice daily from December 1 to 9. On the latter date a single dose of 0.1 gm. was given. From November 15 to December 9 four interval injections of serum, 5 c.c., were given, the last injection being followed within two minutes by convulsions and death. It is of interest that

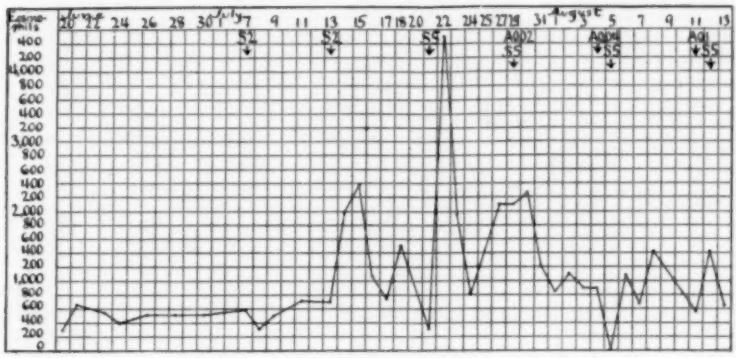

Chart 6.—Experiments on Guinea-Pig 6.

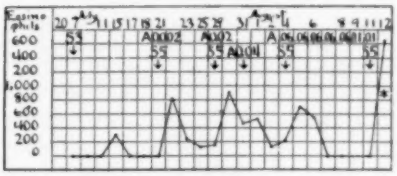

Chart 7.—Experiments on Guinea-Pig 7. * Died.

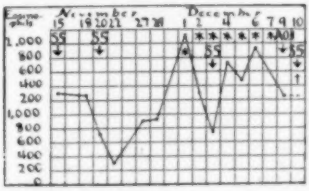

Chart 8.—Experiments on Guinea-Pig 8. * Atropin sulphate, 0.05 gm. twice daily. † Death in two minutes from convulsions.

the large amounts of atropin administered before the fatal injection of serum did not prevent the development of an apparent acute anaphylaxis. The injections of serum were not followed by any notable rise in the number of eosinophils. Throughout the period of administration of atropin and serum the average level of the eosinophil curve remained about as during the control period. December 8 the weight was 543 gm.

EXPERIMENT 9.—Guinea-Pig 9 weighed 521 gm. Control counts showed constant eosinophilia, these cells numbering from 1,000 to 2,000 in a cubic millimeter. Serum, 5 c.c., was given November 15. This was repeated November 20 and was followed by a pronounced rise in eosinophils to 1,825. Atropin, 0.05 gm., was given twice daily from December 1. Serum, 3 c.c., was given December 3 without subsequent eosinophilia. December 10, serum, 5 c.c., was injected. No eosinophilia followed, and on December 11 and the following days these cells diminished in number. A fifth injection of serum, December 16, was followed by a very slight rise in the eosinophils, from zero to 500. In this instance there was definite effect on the eosinophils, the diminution in their numbers after the administration of atropin being constant and striking. The animal showed toxic symptoms and lost weight to 493 gm.

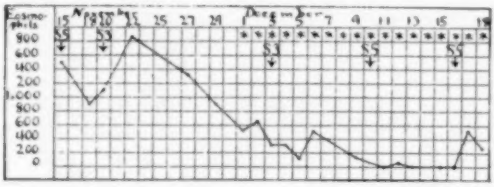

Chart 9.—Experiments on Guinea-Pig 9. * Atropin sulphate, 0.05 gm. twice daily.

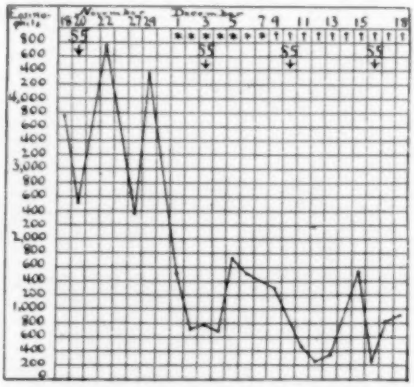

Chart 10.—Experiments on Guinea-Pig 10. * Atropin sulphate, 0.05 gm. twice daily. † Atropin sulphate 0.1 gm. twice daily.

EXPERIMENT 10.—Guinea-Pig 10 weighed 522 gm. Control counts of eosinophils were high—from 2,400 to 4,800. From December 1, atropin, 0.5 gm., was given twice daily. After this date the eosinophils at no time numbered more than 1,700. From December 9 the amount of atropin given was doubled, 0.1 gm. being the dose administered. Following this the eosinophil count fell to 200, and remained constantly low. Interval injections of serum resulted in no significant eosinophilic increase; in fact, in one instance a fall followed such an injection. The effect of atropin seems definite in the case of this animal. There were toxic symptoms and loss of weight to 490 gm.

REVIEW OF EXPERIMENTS

Review of the experiments detailed above brings out the fact that definite fall in the number of eosinophils followed administration of atropin in but two of ten animals. The animals in which such positive result was obtained were two of three receiving large doses of atropin, from 0.05 to 0.1 gm., every twelve hours (Guinea-Pigs 9 and 10). The third animal receiving such doses showed no effect on the eosinophils (Guinea-Pig 8). This guinea-pig, however, died from anaphylaxis before a sufficient period of observation had elapsed to make conclusions of value. A less definite result was seen in Guinea-Pig 1. This animal received daily doses of atropin from 0.03 to 0.2 gm., and showed considerable reduction in the number of eosinophil cells from the high average found throughout the earlier part of the period of observation. Guinea-Pig 2, on the same dosage of atropin, showed little change in number of eosinophils. The smaller doses of atropin, from 0.002 to 0.004 gm., were without influence on the eosinophil content of the blood. In order, therefore, to reduce a high eosinophilia with atropin, very large doses sufficient to cause toxic symptoms must be given at frequent intervals.

INFLUENCE OF ATROPIN ON THE EOSINOPHILIA FOLLOWING INJECTION OF SERUM

Atropin given with, immediately before or twenty-four hours before the interval injection of serum, seemed to inhibit to some degree the subsequent rise in number of the eosinophils (Guinea-Pigs 3, 5, 6, 9 and 10); such rise being absent or less than that occurring in the same animal after injections of serum without atropin. This result is not constant. For exceptions observe Charts 3, 4 and 7. In animals receiving large doses of atropin at intervals of twelve hours, eosinophilic response was slight or lacking (Guinea-Pigs 8, 9 and 10). Frequently there was a drop in the curve of these cells after such injections—a most exceptional occurrence under ordinary conditions.

DISCUSSION OF RESULTS

It seems clear that the mechanism by which the number of eosinophils in the circulating blood of guinea-pigs is increased is not influenced by ordinary amounts of atropin. This mechanism, however, may be depressed or paralyzed by excessive doses of the drug, when such doses are given as often as every twelve hours.

The method of action of atropin in this connection is obscure and involves the question of the general control of leukocytes, which is far from solution. One can only speculate as to the possible influence of atropin on chemotaxis, or as to an altogether hypothetical influence of

the autonomic nervous system on bone-marrow activity which is inhibited by the alkaloid; or one may, perhaps with more reason, consider that the results noted are due to a general interference with a highly specialized function by a strong poison and not to any more specific influence. In connection with the latter conception, Opie's demonstration of the close relation of the number of eosinophils of guinea-pigs to their nutrition is important. Opie[10] found that in guinea-pigs in which there was loss in weight from starvation or other cause the eosinophil cells were regularly much reduced in number. In the present study the nutrition of the animals suffered from the large doses of atropin. Loss of weight and appetite, roughening of the coat, lessened activity, etc., were noted in all animals showing reduction in numbers of eosinophils or lack of response on the part of these cells to serum injections. It may therefore be reasonable to consider the effect of atropin on the eosinophils here observed an indirect one and a result of disturbed nutrition from large doses of a poisonous alkaloid.

There is strong objection to applying by analogy to man conclusions gained by experiments on an animal like the guinea-pig, particularly with atropin, a poison to which the animal is, as compared to man, relatively tolerant. The very large doses of atropin given, however, and the fact that the blood reactions of the guinea-pig are strikingly like those of man, must have weight. Clinically it has never been possible in my experience notably to reduce a hypereosinophilia by atropin in ordinary doses. Animal experimentation seems to lend some degree of corroboration to this clinical experience.

CONCLUSIONS

Atropin in ordinary, non-toxic doses has no striking influence on the eosinophil cells of the guinea-pig's blood.

Given in toxic doses resulting in loss in weight and other signs of disturbed nutrition, and particularly when such doses are repeated as often as every twelve hours, there is a diminution in the number of these cells, and the eosinophilia following interval injections of serum is diminished or absent.

It is probable that this effect of atropin is not specific, but is the depression of a highly specialized function by the general effect on nutrition of large amounts of a strong poison.

CLINICAL OBSERVATIONS ON DEFECTIVE CONDUCTION IN THE BRANCHES OF THE AURICULO-VENTRICULAR BUNDLE*

A REPORT OF TWENTY-TWO CASES, IN WHICH ABERRANT BEATS WERE OBTAINED

EDWARD PERKINS CARTER, M.D.
CLEVELAND, O.

The present communication is based on material collected in the Cardiographic Department of University College Hospital Medical School, London. It includes a description of twenty-two cases in which evidence of defective conduction in one or the other branch of the auriculoventricular bundle was observed electrocardiographically, a description of the characteristic features of aberrant curves and remarks on the clinical associations of the corresponding defects.

As defective conductivity of the main stem of the auriculoventricular bundle gives rise to a characteristic electrocardiogram, so do lesions of its individual branches give rise to abnormal electrocardiographic curves, which, within certain limits, follow definite types. It is with these definitely known forms of abnormal curves that we are concerned.

As *aberrant*[1] are grouped those heart-beats which result as a direct sequence of impulses which arise in a supraventricular focus, but which reach the ventricle either through an unusual route or through normal but partially defective channels. In either case the resulting electrocardiograms will show conspicuous differences from the normal, differences which depend either on a permanent organic lesion, or, less commonly, on a temporary defect in conductivity.

By supraventricular focus is meant a focus in the auricle or junctional tissues above the division of the main stem of the bundle. The impulses giving rise to the independent ventricular rhythm of complete heart-block are of supraventricular origin, for they arise in the main bundle, and so, as we shall see, aberrant types of ventricular contraction may be associated with complete dissociation.

It has been shown by Eppinger and Rothberger[2] that experimental lesions of the branches of the auriculoventricular bundle are followed by characteristic electrocardiographic curves, that a lesion of the right

* Submitted for publication Feb. 19, 1914.

1. Lewis: Heart, 1912, iii, 293.

2. Eppinger and Rothberger: Ztschr. f. klin. Med., 1910, lxx, 1.

branch of the auriculoventricular bundle gives rise, in Lead II (Rt.A.—Lt.L.), to an electrocardiogram which resembles that obtained on stimulation of the left and apical portion of the ventricle, the initial deflection of the ventricular complex being directed downward (apex negative); while a lesion of the left branch of the auriculoventricular bundle gives rise to the reverse picture, an electrocardiogram resembling that obtained on stimulation of the right and basal portion of the ventricle, the initial deflection of the ventricular complex being directed upward (base negative).

In either instance the resulting electrocardiogram is essentially diphasic, and, although always preceded by an auricular complex, the ventricular complexes resemble those known to occur as the result of intrinsic premature ventricular contractions, as shown by the prolongation of the period covering the opening phases, by the increase in amplitude and direction of the initial deflection in the several leads. Soon this experimental evidence was further established by Eppinger and Stoerk,[3] who were able, in two clinical instances by subsequent post-mortem examination, to confirm the diagnosis made from electrocardiograms of a lesion of the right branch of the auriculoventricular bundle.

More recent clinical and electrocardiographic evidence has confirmed these earlier observations, a number of workers having reported in detail cases associated with lesions of one or the other branch of the auriculoventricular bundle, in some of which the diagnosis has been confirmed by histological examination.

Cohn and Lewis[4] report a most unusual case of auricular fibrillation and heart-block, in which it was shown by a histological examination that both branches of the auriculoventricular bundle had been destroyed. The electrocardiograms obtained from this case were practically identical with the curves obtained from a similar experimental lesion by Eppinger and Rothberger.

Lewis[5] has published a report of a unique case illustrating a temporary defect in conductivity of the right branch of the auriculoventricular bundle, the records of which are included in the present series (Case 12).

Mathewson[6] has recently reported four cases associated with lesions of one or the other branch of the auriculoventricular bundle.

In the light of the accumulated observations it has seemed possible to attempt to classify in greater detail the aberrant curves resulting from lesions of the branches of the auriculoventricular bundle.

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3. Eppinger and Stoerk: Ztschr. f. klin. Med., 1910, lxx, 157.
4. Cohn and Lewis: Heart, 1912, iv, 15.
5. Lewis: Brit. Med. Jour., 1913, i, 484.
6. Mathewson: Heart, 1913, iv, 385.

DISTINCTIONS BETWEEN PHYSIOLOGICAL AND ABERRANT TYPES OF CURVE

In order to bring out more clearly the essential distinctions between the normal and the aberrant electrocardiogram, I summarize in tabular form the chief points of difference between them and physiological electrocardiograms. It must be borne in mind, however, that, though we are dealing with an aberrant type of electric curve, the ventricular contractions were of supraventricular origin, each ventricular beat being preceded by an auricular contraction—except in one case of complete heart-block—and that the resulting ventricular complexes alone are abnormal.

In the accompanying figures all three leads have been employed, as it is possible to arrive at accurate conclusions only by an analysis of the curves obtained from the three leads. In Lead II, in the presence of lesions of the branches of the auriculoventricular bundle, the initial deflection of the ventricular complex usually follows the direction of the corresponding deflection in Lead III, but there are exceptions to this rule.

TABLE 1.—CONTRASTS BETWEEN NORMAL AND ABERRANT ELECTROCARDIOGRAMS

NORMAL	ABERRANT
1. Supraventricular complex. Presence of auricular or *P* summits.	1. Supraventricular complex. Presence of auricular or *P* summits.
2. *P-R* interval .13 to .18 second, never more than 0.2 second.	2. *P-R* interval frequently prolonged beyond 0.2 second.
3. *Q-R-S* interval less than 0.1 second, and less than one-third of entire complex.	3. *Q-R-S* interval exceeds 0.1 second and as a rule constitutes more than one-third of the entire complex.
4. Relatively small amplitude of initial deflections.	4. Relatively increased amplitude of initial deflections.
5. Final deflection *T* upright and in the same direction as the most prominent deflection (*R*) in Leads I and II, and usually in Lead III.	5. Final deflection *T'* usually in a direction opposite to that of the prominent initial deflection. May be upright or inverted.
6. Initial deflections as a rule unnotched.	6. Initial deflections almost always show notching in one lead at least. Many bizarre forms seen.
7. Final deflection *T* as a rule plainly to be seen, but not exaggerated.	7. Final deflection *T'* frequently much exaggerated.

Thus it will be seen that the differences between the normal and the aberrant electrocardiogram consist essentially in the longer duration of the opening phases, in the greatly increased amplitude of the initial deflections, in the notching of R' and S' so often seen in the aberrant curves—the initial deflections are spoken of as R' and S', to distinguish them from the deflections R and S of the normal curve—and in the generally diphasic character of the aberrant curve, especially in Leads I and III, while the normal electrocardiogram is triphasic or quadriphasic.

Whatever the location of the lesion, whether involving the right or the left branch of the auriculoventricular bundle, the prominent initial deflections are always in *opposite* directions in Leads I and III. This rule, while constant for R' and S', usually holds for the final deflection T', which, as has been stated, commonly lies in a direction opposite to the prominent initial deflections R' and S', and is usually conspicuous.

Figure 8 illustrates in detail the distinguishing characteristics of the aberrant electrocardiogram; it is an instance of temporary defect in the conductivity of the right branch of the auriculoventricular bundle, associated with relative left ventricular hypertrophy. The greatly increased duration and amplitude of the initial deflections is at once apparent. The *P-R* interval measures 0.18 sec. The *Q-R-S* interval measures more than 0.1 sec. The conspicuous initial deflections R' and S' in Leads I and III are seen to be in opposite directions. The initial deflection S' in Lead II follows the same direction as the corresponding deflection in Lead III. The final deflection T' is in a direction opposite to the conspicuous initial deflection in all leads, being inverted in Lead I, and upright in Leads II and III. The notching of R' in Lead I and of S' in Leads II and III is apparent. The final deflection T'' is much exaggerated.

DISTINGUISHING POINTS BETWEEN THE ABERRANT ELECTROCARDIOGRAM AND THAT OBTAINED AS A RESULT OF CARDIAC HYPERTROPHY

While it is freely admitted that the attempt to correlate certain types of electrocardiogram with a definite form of cardiac hypertrophy may need subsequent revision, it has been shown that in the presence of specific valve-lesions, commonly associated with preponderating hypertrophy of the left or right ventricle, distinctive electrocardiograms have been obtained, controlled in a small number of instances by subsequent post-mortem examination.[7]

It may, therefore, be stated that the electrocardiographic curves obtained as a result of cardiac hypertrophy, from whatever cause, depend essentially on the degree and distribution of the hypertrophy present in a given case, whether left or right side predominates.

In the presence of left ventricular preponderance the resulting electrocardiogram is characterized by a tall, conspicuous initial deflection R in Lead I, with an inconspicuous S, and by a deep deflection S, with an inconspicuous R, in Lead III. Lead II is less characteristic.

In the presence of right ventricular preponderance the picture is reversed. In Lead I, R is inconspicuous, the initial deflection S appearing as a deep depression, while in Lead III, R appears as a tall conspicuous peak, and S is small.

7. Lewis: Clinical Electrocardiography, London, 1913, p. 24.

It is apparent that, as in the aberrant electrocardiogram, the most prominent initial deflections in Leads I and III are in opposite directions, the direction depending on the predominance of one or the other ventricle. The electrocardiogram obtained from a case of simple cardiac hypertrophy may be distinguished from the aberrant type, which it resembles in many respects, by the shorter duration of the opening phases of the complex, by the lesser amplitude of the prominent deflections, by the direction of the deflection T in relation to the deflections R and S, by the absence of notching of R and S, and by the normal triphasic or quadriphasic character of the complex. In aberrant curves T' is in the opposite direction to the main initial deflection (R' or S') in Leads I and III; in the curves of hypertrophy T and R or S are often in the same direction in these leads.

When, as occasionally happens, a temporary defect in conductivity occurs in the course of an acute breakdown of an already hypertrophied heart, a condition almost always present in these cases, one may be able to compare the aberrant curve obtained from such a heart with the curve of simple hypertrophy obtained later, after the disappearance of the transient functional defect.

Such instances are not common, but the occurrence of a transient aberrant electrocardiogram is most important from the point of view of a comparison of the aberrant curve with the physiological type in the same case.

Allusion has been made to the single instance of such temporary defect in conductivity included in this series; and the facts stated above are well illustrated by a comparison of Figures 8 and 9, which accompany Case 12, the bizarre aberrant electrocardiogram resulting from the acute lesion of the right branch of the bundle, giving place, on the subsidence of the acute process, to the physiological electrocardiogram recorded on the following day.

No change could be more marked: the earlier curve due to defective conduction plus left ventricular preponderance, the latter essentially the curve of simple cardiac hypertrophy. No more striking evidence than these two figures could be offered that the aberrant electrocardiogram does not depend on the presence of cardiac hypertrophy.

In this connection reference may also be made to Case 20, Figures 16 and 17. Here we are fortunate in having obtained the physiological electrocardiogram of the individual, before the development of defective conductivity, for comparison with the curves obtained later and after the development of the lesion involving the right branch. The difference between the two figures is apparent at a glance: the earlier physiological electrocardiogram could not by any possible chance be confused with the aberrant complex obtained later, after the development of defective conductivity.

SIDE OF THE DEFECT

In the twenty-two cases of this series the right branch of the auriculoventricular bundle has been involved in twenty-one, the left branch in but one instance. The majority of previously described cases also are those in which a defect of the right branch has been diagnosed.

TABLE 2.—SUMMARY OF CARDIOGRAMS IN CASES 1 TO 22 *

Patient	Case No.	Fig. No.	*P-R* Interval Seconds
D. B.	1	.....	0.13.
G. S.	2	.....	0.24.
J. P.	3	.....	A. F.
H. C.	4	1	0.20.
J. P.	5	2	A. F.
R. S.	6	.....	0.34 (1-17-11).
			0.30 (8-7-12).
			C. H. B. (11-3-12).
			Dropped beats (11-13-12).
		3	Dropped beats (2-6-13).
		.....	C. H. B. (11-24-13).
W. S.	7	4	0.21 (three observations).
H. C.	8	.....	0.29.
S. R.	9	5	0.17-0.18.
R. B.	10	6	0.21-0.23 (four observations).
C. H.	11	.....	0.26 (6-12-12).
			0.16 (7-12-12).
		7	0.20 (7-25-12).
W. C.	12	8	0.18 (8-18-12).
		9	0.18 (8-19-12).
		.....	0.20 (10-6-12).
R. A.	13	10	0.13.
W. P.	14	.....	A. F.
J. W.	15	11	0.20.
D. E.	16	12	0.14.
W. O.	17	.....	0.28 (2-17-13).
		13	0.17 (5-12-13).
G. C.	18	14	0.20.
W. J.	19	15	0.18-0.20 (two observations).
D. H.	20	16	0.30 (2-27-13).
			0.24 (3-5-13).
			0.24 (4-7-13).
			0.21 (4-10-13).
			0.24 (4-28-13).
		17	0.22 (5-5-13).
		.....	0.29 (5-15-13).
J. M.	21	18	0.17.
E. N.	22	19	C. H. B.

* The conditions found in Cases 1 to 22 are as follows: Aortic disease, 7, 9, 10, 11, 17, 18, 20, 21; varying degrees of heart-block, 2, 4, 6, 7, 8, 10, 11, 12, 15, 17, 18, 20, 22; Cheyne-Stokes respiration, 2, 3, 5, 16; auricular fibrillation, 3, 5, 14; various other associated pathological states, 1, 4, 8, 13, 15, 19, 22. Subsequent histories are: Dead, Patients 2, 3, 5, 7, 9, 10, 11, 14, 16, 18, 19, 20; alive (condition precarious) 8, 12, 13, 17, 21, 22; alive (condition not precarious) 1 and 4; history unknown (condition precarious when last seen) 6 and 15.

There is no entirely satisfactory explanation of this difference in incidence. It is possible, however, that the predominance of the right-side lesion may be explained on anatomical grounds, the right branch, as a circumscribed track, being by far the longer.

Table 2 summarizes the cases reported in detail. In the column giving the *P-R* intervals, these have been grouped in chronological order; otherwise, so far as is possible, wholly on the basis of the associated pathological condition.

RELATION TO AURICULOVENTRICULAR BLOCK

Deficiency in the conduction of the branches of the bundle has a close relation to a similar deficiency in the main stem of the auriculoventricular trunk. It has been emphasized by several writers, Sternberg[8] and Monckeberg[9] among others, that the conducting tissues which unite the auricles and ventricles have a more or less special pathology. Aschoff[10] has called attention to their liability to damage from small hemorrhages, as a result of their curious sheathing with connective tissue; and it is known that fatty degeneration falls unequally on the conducting tissues and the rest of the muscle of auricle and ventricle. Aschoff[11] and Butterfield[12] have shown that the infiltration in rheumatic carditis is observed in the subendocardial tissues and that it is especially prone to invade the conjunctional tissues. That the conducting tissues have a special physiology is certain, for their glycogen content is known to be much greater than that of ordinary cardiac muscle (Nagayo[13]).

Dealing with the same question from a purely clinical point of view the facts point clearly in the same direction. The normal human *P-R* interval usually varies between 0.13 and 0.18 seconds; a higher value than 0.18 is very exceptional; the commonest values are from 0.13 to 0.16 seconds, as shown by Lewis and Gilder.[14] In the present series of twenty-two cases, a sequential rhythm has been present in eighteen. In thirteen of these eighteen cases the *P-R* interval was prolonged, constantly or on occasion. In four (Cases 6, 12, 17 and 20) it showed considerable variation. One of these (Case 6) showed auriculoventricular heart-block of extraordinarily varying degree, namely, simple prolongation of the interval, dropped beats and complete dissociation. In another of the series (Case 22) complete dissociation was a constant feature.

8. Sternberg: Verhandl. d. deutsch. path. Gesellsch., 1910, xiv, 102.
9. Monckeberg: Verhandl. d. deutsch. path. Gesellsch., 1912, xvi, 460.
10. Aschoff: Verhandl. d. deutsch. path. Gesellsch., 1910, xiv, 3.
11. Aschoff: Brit. Med. Jour., 1906, ii, 1103.
12. Butterfield: Heart, 1912, iii, 203.
13. Nagayo: Verhandl. d. deutsch. path. Gesellsch., 1908, xii, 150.
14. Lewis and Gilder: Phil. Tr. Roy. Soc., 1912, ccii, 351.

This clinical evidence speaks very strongly for the frequency of functional damage of the conducting tissues as a whole, though necessarily the damage varies in extent in different parts of the system from case to case. In many of the published cases of complete heart-block, trunk-branch lesions are demonstrated by the electrocardiographic curves.[15]

Experimental evidence points in the same direction; Lewis and Mathison[16] have shown that asphyxia of cats produces auriculoventricular heart-block, and that at the same time it frequently damages the conduction in the main branches of the auriculoventricular system.

We have every reason to conclude, from the evidence derived from all sources, that inflammatory lesions and invasions of the heart by poisons may act in a more or less specific manner, picking out the special system of muscle fibers which unite the auricle and ventricle. The physiology and the pathology of these tissues is largely a special physiology and pathology.

RELATION TO AORTIC VALVE DISEASE

It is a remarkable fact that out of the present series of twenty-two cases, eight patients have exhibited well-developed aortic valve lesions. The frequent association of damage of the branches of the auriculoventricular bundle with aortic valve disease can not be regarded as purely accidental. Yet the association meets with no very plausible explanation; especially so because in each of the eight cases the right trunk was defective. Lesions of the aortic valve would be expected, *a priori,* in association with lesions involving the conductivity of the left branch, for the bases of the valves are in close proximity to it. Nevertheless this association does not occur. Too much emphasis may be laid on this argument; it is weakened by a knowledge of the relative frequency of lesions of the right and left trunk-branches; the former are far commoner. In the present series the right branch was defective in twenty-one cases, the left in one only.

An explanation that the curves are in reality but an expression of left-side muscular preponderance does not suffice. It is perfectly true that the curves of the aberrant type, associated with right-branch lesions, and those speaking for left-side preponderance resemble each other; but there are, nevertheless, clear and recognizable distinctions between them, distinctions which have been emphasized already and which are to be seen in the curves of Cases 12 and 20.

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15. See the case reported by Cohn and Lewis, already referred to, and the second case of Mathewson's series, to which reference has been made.

16. Lewis and Mathison: Heart, 1910, ii, 47.

We are able to produce very satisfactory evidence that the curves in question are not those of left-side preponderance pure and simple, for in these two instances the physiological electrocardiograms for the particular individuals have been obtained for comparison. In Case 12, the change in the type of electrocardiogram is profound and sudden; the aberrant type of curve (Fig. 8) could not result purely from hypertrophy, when within a day an entirely different and more normal type of curve is displayed (Fig. 9). The reason for the frequent association of aberrant curves with aortic valve disease is, therefore, still obscure.

RELATION TO EXTRASYSTOLES

The common occurrence of extrasystoles in conjunction with aberrant forms of electrocardiograms suggests that the lesion which produces the conduction defect may also awaken the new impulses; this the more, since it has been suggested that extrasystoles are often of bundle or bundle-branch origin. It is necessary to examine the direction of the deflections from this point of view. Our curves give us only meager information. Though extrasystoles were seen in a much larger proportion of the cases, the curves in our possession show them in seven instances only. We consider Leads I and III as of most importance from our present point of view. The aberrant curves are mainly diphasic; the curves due to extrasystoles are also mainly diphasic, but in a given case the deflections of the diphasic complexes are usually in opposite directions when aberrant and extrasystolic curves are compared (Table II).

That is to say, in most cases the extrasystole arises in the ventricle opposite to that in which the aberrant beat originates. This is what would be expected if a lesion produced defective conduction in the branch, and also provoked extrasystoles from it. Thus in Cases 10, 14 and 22 the extrasystoles arose from the right or basal portion of the heart, as shown by all leads. In Case 3 the extrasystoles were, on the other hand, from the left and apical portion of the ventricle. In the remaining Cases 11, 15, 20, the deflections of the extrasystole were opposite to those of the aberrant curve in one lead (Lead II), but in the same direction in another lead (Lead I or Lead III).

RELATION TO PROGNOSIS

The prognostic value of any given sign in disease of the heart is always a difficult problem. Especially is this the case when the sign is frequently associated with other signs of a grave nature.

An organic lesion confined to one branch of the auriculoventricular bundle is hardly one which can be regarded as jeopardizing life. We

find that a bundle-branch lesion may be present for many years and the heart still show general efficiency (Cases 1 and 4). But just as lesions of the branches are rarely confined to the branches, but are associated with defects in the main bundle, so too the defects are probably but rarely confined to the conducting system, but spread beyond it into the general musculature to a lesser or greater extent.

From analogy with auriculoventricular heart-block, we must regard a bundle-branch lesion as significant of an invasion of the heart-muscle, be it acute or chronic, and therefore of considerable gravity in most instances. In the present series of cases, the gravity of such a lesion is well illustrated. In twenty-two cases, reports of which have been

TABLE 3.—SUMMARY OF CARDIOGRAMS IN CASES 1 TO 22 SHOWING OCCURRENCE OF PREMATURE BEATS *

Case No.	Direction in Relation to Initial Deflections	Lead
3	Same	I, III
10	Opposite	I, II, III
11	Opposite	II
11	Same	III
14	Opposite	I, II, III
15	Same	III
20	Opposite	II
20	Same	I
22	Opposite	I, III

* In cases 2, 16, 17, 21, premature beats were present, but were not recorded electrocardiographically.

collected within a few years, twelve patients are known to have died (Patients 2, 3, 5, 7, 9, 10, 11, 14, 16, 18, 19, 20). Six are alive but known to be in a precarious condition (Patients 8, 12, 13, 17, 21, 22); two have been lost sight of, but were in a precarious condition when last seen (Patients 6 and 15). Two only (Patients 1 and 4) have been under observation for considerable periods and show no signs of increasing heart-failure.

The frequent association of the condition with alternation of the heart, auricular fibrillation (Cases 3, 5, 14), Cheyne-Stokes respiration (Cases 2, 3, 5, 16), great enlargement of the heart and signs of circulatory embarrassment is in itself significant.

REPORT OF CASES

CASE 1.—*Myocarditis, fibrosis of the left lung with bronchiectasis.*

Patient.—D. B., a girl, aged 16, was admitted to the hospital during the year 1906, complaining of cough, shortness or breath and pain in the left side of the chest.

Family History.—A sister has had rheumatic fever. Her father died of pneumonia complicated by "heart disease."

Personal History.—A delicate child at birth, the patient has had a cough all her life; though it has become much more troublesome of late. She has had no acute illnesses. There is no history of rheumatic fever or chorea. The shortness of breath, especially prominent on exertion, and the pain in the chest are recent developments. She brings up a little yellow sputum.

Condition on Admission.—The patient is poorly developed, with a certain degree of scoliosis, the curvature being toward the left side. The color of the cheeks is heightened. There is impairment of resonance over the whole left side of the chest both in front and behind; the capacity of this side is diminished. In the left intrascapular region the breath-sounds are tubular and bronchophony and pectoriloquy are heard; coarse clicking râles are audible over the same region, some of which synchronize with the heart-beats. A little purulent sputum contained no tubercle bacilli. The respirations are normal.

Heart.—Pulsation is seen over almost the whole left chest in front. The point of maximum impulse is diffuse in the sixth interspace near the posterior axillary line; posteriorly it is thrusting in character. A diastolic thrill is felt over the apex region. The cardiac dulness extends from the third rib above, and from the left margin of the sternum to the posterior axillary line. Systolic and diastolic murmurs are heard at the apex. A harsh systolic murmur is present at the aortic cartilage. The pulse is 76 to the minute and regular; the arteries are soft. There are no signs of venous engorgement or of dropsy. The urine contains no albumin.

Condition on Readmission (Nov. 9, 1912).—After six years the patient has been readmitted for the same symptoms. Pain has been present for twelve months, and shortness of breath, which has never entirely abated, is more distressing. From time to time she brings up large quantities of sputum. The physical signs at this time are very similar to those previously observed. The signs over the fibrosed lung are almost unaltered. The limits of cardiac dulness are the same. The murmurs remain unchanged. The respirations are not increased; there is but little venous engorgement; dropsy is absent; the urine contains no albumin. The temperature never rises above 99 F.

Electrocardiograms.—The P-R interval measured 0.13 second. In Leads I and II the initial deflection was abrupt and notched, having a total duration of 0.1 second. In Lead III an upward phase was succeeded by a larger downward phase; the two together measuring 0.1 second.

Subsequent History (June 21, 1913).—The patient is alive and in fair condition.

CASE 2.—*Myocarditis, extrasystoles, alternation, Cheyne-Stokes breathing and death.*

Patient.—G. S., a business man, aged 63, was first seen April 27, 1910, complaining of cough and shortness of breath on exertion.

Personal History.—With the exception of occasional attacks of gout the patient has had no illness since his boyhood. He has complained of his present symptoms for the past five months; these consist chiefly of an irritating cough, which is accompanied by considerable shortness of breath.

Condition on Admission.—The patient is well developed and shows no sign of cyanosis. He has Cheyne-Stokes breathing.

Heart.—The cardiac dulness extends two inches to the left of the nipple line. The first sound at the apex is reduplicated. There are no murmurs. The pulse is full and intermits from time to time. The blood-pressure is 125 mm. Hg.

Graphic Records.—Polygraphic records showed that the intermissions of the pulse were the result of premature contractions of the ventricle. These are followed by alternation. The *a-c* interval is distinctly prolonged.

Subsequent History.—The patient died twelve months later.

Electrocardiograms.—The P-R interval measured 0.24 second. The ventricular curves were similar to those shown in Figure 8.

CASE 3.—*Rheumatic myocarditis, high blood-pressure, auricular fibrillation, alternation, Cheyne-Stokes breathing, nephritis, urethral stricture; graduat failure and death.*

Patient.—J. P., a shop-keeper, aged 65, was admitted to the hospital Aug. 1, 1911, complaining of shortness of breath, pain in the chest, loss in weight and sleeplessness.

Personal History.—At the age of 26, 32 and 48 the patient had rheumatic fever. At 59 he had a slight "apoplectic stroke" and lost the sight of his left eye. He has been treated for urethral stricture for eighteen years.

Present Illness.—The patient states that twelve months ago he was quite free from any discomfort. About this time he began to suffer from shortness of breath, which has steadily increased; at present the slightest exertion brings it on. He has experienced shooting pains in the left side of the chest, and also, from time to time, in the right hypochondrium. The breathing is said to be heavy and periodic at night. He has lost 42 pounds in weight. He sleeps but two or three hours each night. His appetite is poor.

Condition on Admission.—The patient is well nourished and cyanotic and exhibits conspicuous Cheyne-Stokes breathing. The liver is palpable and tender an inch below the costal margin. There is slight edema of the legs and some ascites present. The specific gravity of the urine ranges from 1.010 to 1.016; albumin, sugar and pus are present in excess. The pulse is very irregular, the rate being 90 to 120 per minute; the arteries seem but little thickened. Most of the pulse beats pass a cuff-pressure of 200 mm. Hg.

Heart.—The apex-beat is not visible; the limits of cardiac dulness lie 2 inches to the right and 5 to the left of the midsternal line. The heart-sounds are clear, but very irregular; there are no murmurs.

Course of Disease.—The patient remained in the hospital for some weeks and improved a good deal in health. He passed from observation, but his wife wrote subsequently to say that he gradually became weaker, thinner and more short of breath. Dec. 5, 1911, when sitting up in a chair, he asked to be put to bed again, but almost immediately he became relaxed and died.

Electrocardiograms.—The P-R interval could not be determined, owing to the constant presence of auricular fibrillation. The ventricular complexes were similar to those shown in Figure 6. The extraventricular systoles recorded arose from the left and apical portion of the heart, being in the same direction as the initial deflections *R'* and *S'* in Leads I and III.

CASE 4.—*Pericarditis with effusion, arhythmia, drainage of the pericardium and recovery.* (Electrocardiograms, Fig. 1.)

Patient.—H. C., a woodcarver, aged 28, was admitted to the hospital Aug. 8, 1911, complaing of indigestion and palpitation of the heart.

Family History.—Negative.

Personal History.—The patient had pneumonia as a child and so-called "muscular rheumatism" two years ago. There is no history of rheumatic fever or chorea. He denies syphilis.

Present Illness.—For the past eight weeks the patient has complained of indigestion and palpitation of the heart. Four weeks ago, July 12, he had a severe attack of vomiting, and has had frequent similar attacks since this time. Since the onset of these attacks of vomiting he has been quite breathless on slight exertion, and latterly has noticed that there was some swelling of his legs.

Condition on Admission.—Edema of the legs and scrotum and well-marked signs of compression of the left lung are present. The liver is palpable at the

umbilicus. The temperature is 99.8 F. The respirations are from 28 to 32 per minute.

Heart.—The upper limit of cardiac dulness lies at the first interspace, to the right 3¾ inches and to the left 8 inches from the midsternal line. Pulsation over the precordium is very slight. There is a distant soft systolic murmur heard over the region of the apex. The first and second sounds are heard everywhere, but are very distant. There is a pleuropericardial friction-rub heard in the third and fourth left interspaces.

Course of Disease.—Aug. 13, 1911: The fourth left costal cartilage was removed, the pericardium opened and a pint of hemorrhagic fluid withdrawn.

Aug. 18, 1911: For several days the patient has had irregularity of the heart, the result of fibrillation of the auricles. To-day the pulse is regular, the *a* wave has appeared again.

Aug. 25, 1911: The cardiac dulness has been greatly reduced by tapping.

Sept. 14, 1911: The patient was tapped again to-day and 2½ pints of blood-stained fluid were removed.

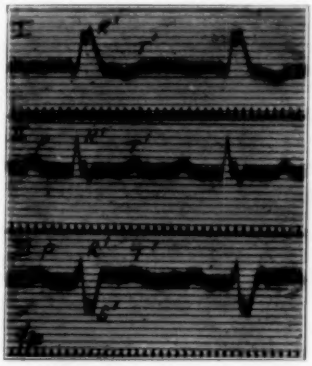

Fig. 1.—Electrocardiograms. Case 4. The *P-R* interval measures 0.2 second. The *Q-R-S* interval is increased, measuring 0.13 second. In Lead I, the initial deflection *R'* is conspicuous as a broad summit with a markedly split apex. The final deflection *T'* is inverted, lying in a direction opposite to the prominent summit *R'*. In Lead II, the initial deflection *R'* appears as a conspicuous peak with marked notching. The final deflection *T'* is upright, lying in the same direction as *R'*. In Lead III, *R'* is present, but *S'* appears as a prominent broad depression with marked notching of the base. The final deflection *T'* is upright, lying in a direction opposite to the prominent deflection *S'*. The increased duration of the opening phases of the complex, the conspicuous notching, and the direction of the final deflection *T'* in relation to prominent deflections *R'* and *S'*, are apparent.

Sept. 26, 1911: A note was made as follows: The pericardium is filling again. The pulse has remained almost continuously regular. On one or two occasions the patient has noticed some palpitation of the heart. The temperature has been normal throughout, except for a few days after the operation.

Sept. 11, 1912: The patient was seen on this date and is apparently in very fair condition. There is very little breathlessness. The limits of cardiac dulness are considerably reduced and no effusion is demonstrable in the pericardium.

Subsequent History.—July, 1913: The patient is alive and apparently in good condition.

CASE 5.—***Cardiac hypertrophy, myocarditis, auricular fibrillation, arteriosclerosis, Cheyne-Stokes respiration, cardiac failure and death.*** (Electrocardiograms, Fig. 2.)

Patient.—J. P., a hotel-keeper, aged 69, was admitted to the hospital Nov. 11, 1911, complaining of breathlessness, feelings of faintness and swelling of the legs.

Family History.—Negative.

Personal History.—The patient remembers no past illness. He has not had rheumatic fever and denies syphilis. He has drunk beer in moderation, but has been a heavy smoker.

Present Illness.—In October, 1907, the patient met with an accident, which he states injured his spine. He was laid up at this time some weeks, but made a good recovery, though he insists that his heart has been weak since the mishap. In October, 1911, he contracted a severe cold and had a slight pneumonia, being ill in bed for some weeks. From this time his symptoms have increased. At present he complains of great breathlessness on slight exertion, general lassitude and frequent attacks of dizziness. Recently his legs have become swollen and he has noticed puffiness about the eyes.

Condition on Admission.—The patient is well built, but exhibits conspicuous breathlessness. There is a slight degree of cyanosis present. The legs are edematous; the liver is not palpable; the flow of urine is abundant and albumin is excessive. The pulse is very irregular and weak (auricular fibrillation), rate 80 per minute. The arteries show thickening; the systolic blood-pressure is 160 mm. Hg (strongest beats).

Heart.—The heart's impulse is not visible; the limits of dulness are difficult to ascertain as the lungs encroach so much on it. The heart-sounds are faint; the first sound is reduplicated; no murmurs are heard.

Course of Disease.—During the patient's stay in the hospital Cheyne-Stokes breathing was often observed; he became delirious from time to time, and on occasions was in a state of coma. He was treated with digitalis and, though his life was despaired of, he was eventually discharged a good deal improved.

Readmission.—A year later he was again admitted with the same symptoms.

Nov. 1, 1912: The legs are again swollen, he has much cough and breathlessness and sleeps badly. His symptoms have been increasing for the past three weeks and he has lost in weight. The physical signs are much the same as on his first admission.

The heart is still irregular, but of normal rate. The upper limit of cardiac dulness lies at the third interspace, to the right 1½ inches and to the left 5½ inches from the midsternal line. There are no murmurs; the first sound is faint, but not reduplicated. The liver is just palpable.

Course of Disease.—Cheyne-Stokes respiration was observed on many occasions and he was sometimes delirious at night. Although constantly breathless cyanosis was never prominent. He again so far recovered as to be able to leave the hospital much improved.

Readmission (March 3, 1913). (Note T. L.) The patient lies propped up in bed. His lips are pale and very slightly cyanotic. His breathing is labored and from time to time shows periodicity in depth. There is slight edema of the ankles. The pulse-rate is 84 to the minute and irregular (auricular fibrillation and defect of the right bundle branch as before). The signs in the heart remain unaltered. There is impairment of resonance at the bases of both lungs. The liver is easily palpable below the costal margin.

March 5, 1913: The respirations are irregular and show periodicity in depth. No apneic periods, however, are present.

March 19, 1913: There is to-day a good deal of improvement in the patient's breathing; it is easy and almost natural during the day, but tends to show a periodicity at night.

March 21, 1913: The patient was discharged to-day in a much improved condition. There was at this time no breathlessness if he moved slowly, and his

color was good. During his stay in the hospital the temperature was normal. The systolic blood-pressure ranged between 130 and 140 mm. Hg. The urine varied in quantity between 1,100 and 2,500 c.c. per diem. The reaction was acid; the specific gravity ranging from 1.010 to 1.012, except just after admission, when it was 1.022; albumin, blood and casts were not found.

Readmission (April 3, 1913).—Seen in the out-patient's department on this day, the patient was evidently much worse and was readmitted to the hospital. He has been voiding very little urine and what is passed is dark in color. He is considerably, but not deeply, cyanosed and is very dyspneic. The breathing is rapid, 32 to the minute, and labored, while he lies well propped up. The liver is again large, and there is impairment of resonance at the bases of both lungs and numerous crepitations are heard over the whole chest. The signs in the cardiovascular system are unaltered.

April 6, 1913: Cheyne-Stokes breathing with long apneic pauses is observed at night. Patient is still cyanotic. Electrocardiograms were taken on this date.

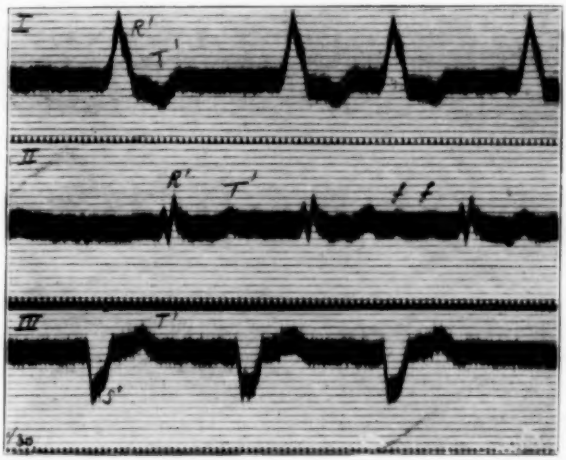

Fig. 2.—Electrocardiograms, Case 5. The *P-R* interval could not be determined owing to the constant presence of auricular fibrillation. The *Q-R-S* interval is increased, measuring 0.16 second. In Lead I, the increased amplitude and the marked notching of the initial deflection *R'* are conspicuous. *T"* is inverted lying in a direction opposite to the summit *R'*. In Lead II, *R'* shows marked splitting. *T"* is upright lying in the same direction as the deflection *R'*. In Lead III, the broad, markedly notched deflection *S'*—in a direction opposite to the summit *R'* in Lead I—is conspicuous. *T"* is upright lying in a direction opposite to the initial deflection *S'*. The diphasic character of the ventricular complex is apparent in Leads I and III.

April 7, 1913: The dyspnea is still conspicuous. In the morning 30 ounces of urine were withdrawn by catheter and a little albumin was found. An attempt was made to bleed the patient, but the flow was so poor that it had to be abandoned. A urea estimation gave 2.3 gm. per liter of blood. (Cotton.) Death occurred without further warning the same day, the patient collapsing, becoming very cyanotic and the respirations ceasing after a few forcible inspiratory efforts.

During his last illness the amount of urine varied between 100 and 900 c.c. per diem. It was dark in color, of acid reaction, the specific gravity ranging between 1.020-1.030, and was loaded with urates; once only a faint trace of albumin was found. The systolic blood-pressure varied between 116 and 138 mm. Hg. The temperature was subnormal throughout.

Post-Mortem Examination (April 8, 1913).—Deep cyanosis. Dropsy of the legs. The pleural cavities each contain about one pint of clear fluid. There is an excess of fluid in the pericardial and peritoneal cavities. The lungs are slightly emphysematous; the bases are edematous and congested. The heart is large and covered with fat; the muscle shows yellow striation. The cavities are dilated and the walls seem hypertrophied. The valves are normal. No antemortem clots were found. The aorta and large arteries show generalized arteriosclerosis with calcareous deposits; the aortic arch is dilated. The liver is large, fatty and congested. The splenic pulp is friable and dark in color. The kidneys are congested; the capsules strip off leaving a slightly torn and granular surface; there are a few scars on the surface, but no cysts. The cortex and medullary substance show narrowing. The brain is normal though the membranes over the vertex are edematous; the remaining organs are normal.

Electrocardiographic curves taken on repeated occasions always showed the same mechanism, namely, fibrillation of the auricles and absent conduction in the right branch of the auriculoventricular bundle. The ventricular rate was always slow, ranging from 60 to 90. (See Fig. 2.)

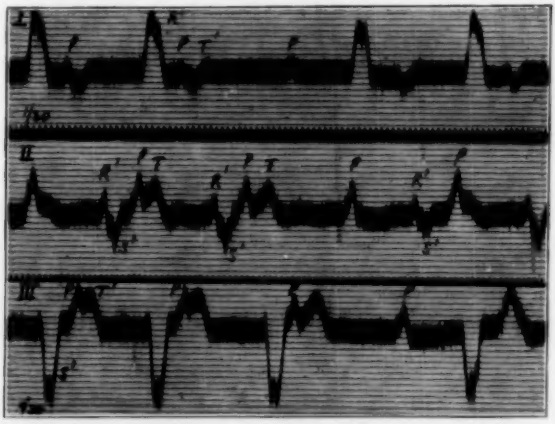

Fig. 3.—Electrocardiograms, Case 6. Note the degree of heart-block present. From time to time a failure of response to the previous auricular contraction is shown. In Lead I, the initial deflection *R'* appears as a conspicuous summit of increased amplitude and duration, with marked notching. The final deflection *T'* is inverted, lying in a direction opposite to the prominent *R'*. The gradual increase in the *P-R* interval up to the point of the occurrence of the dropped beat is apparent. There is no ventricular response to the second *P* summit in this lead. In Lead II, *R'* is present, but the deflection *S'* appears as a conspicuous depression of increased amplitude and duration with marked notching. The final deflection *T'* is upright, lying in a direction opposite to the prominent *S'*. Here again the failure of a ventricular response, following the second auricular contraction, is apparent. In Lead III, *R'* is absent. The initial deflection *S'* appears as a depression of greatly increased amplitude and duration, with conspicuous notching. The final deflection *T'* is upright, lying in a direction opposite to the prominent *S'*. There is a missed ventricular response after the third *P* summit.

CASE 6.—*Cardiac hypertrophy, mitral disease, paroxysmal tachycardia and auriculoventricular heart-block in varying degrees.* (Electrocardiograms, Fig. 3.)

Patient.—R. S., a bootmaker, aged 64, was admitted to the hospital Dec. 1, 1911, complaining of shortness of breath, attacks of faintness and pain in the chest.

Personal History.—Twelve years ago the patient suffered from a fistula *in ano*. There is no history of any other illness. He denies syphilis. He has been a moderate user of alcohol and tobacco.

Present Illness.—Twelve months ago he first noticed shortness of breath on exertion. For six weeks he has experienced attacks of pain in the chest accompanied by faintness. In some of these attacks he vomits, and on one occasion he lost consciousness. His pulse-rate in the attacks, which were witnessed by his physician, Dr. Alex. Brown of Kentish Town, rose to at least 250 per minute.

Condition on Admission.—The patient is well nourished and shows no sign of breathlessness and little or no cyanosis. The liver is enlarged. There is no dropsy. The temperature is normal. The pulse is regular, its rate being from 72 to 144 per minute; the vessel-wall is thickened. The systolic blood-pressure varies between 64 and 130 mm. Hg.

Heart.—The heart's impulse is neither visible nor palpable. The limits of cardiac dulness lie at the fourth rib above, to the right at the left sternal margin, and to the left in the fifth space four inches from the midsternal line. There is a blowing systolic murmur at the apex; and also a systolic murmur at the aortic cartilage.

Subsequent History.—Unknown.

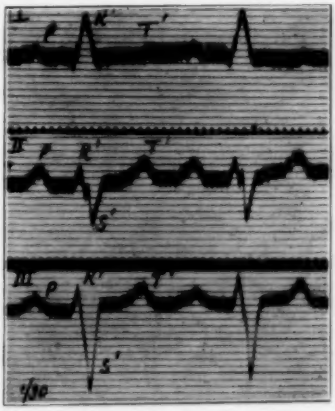

Fig. 4.—Electrocardiograms, Case 7. The *P-R* interval measures 0.21 second. The *Q-R-S* interval measures 0.11 second. In Lead I, the initial deflection *R'* appears as a peak of increased duration and slight increase in amplitude, with conspicuous notching. The final deflection *T'* is upright, lying in the same direction as the initial *R'*. In Lead II, *R'* is present but inconspicuous. The deflection *S'* appears as a depression of increased duration with marked notching. The final deflection *T'* is upright, lying in a direction opposite to *S'*. In Lead III, the initial deflection *R'* is again inconspicuous. The deflection *S'* appears as broad depression of greatly increased amplitude. The final deflection *T'* is upright, lying in a direction opposite to the prominent *S'*. The increase in the *P-R* interval is apparent in all three leads.

Electrocardiograms.—This case is of especial interest because of the presence of varying degrees of auriculoventricular heart-block, which were present at different times; the patient having been under observation over a long period before his entrance into the hospital. (Fig. 3.) Jan. 17, 1911, the *P-R* interval measured 0.34 second, and Aug. 7, 1912, 0.3 second. Nov. 3, 1912, complete auriculoventricular heart-block was present. Nov. 13, 1911, dropped beats, Feb. 6, 1913, dropped beats, and again Feb. 24, 1913, complete auriculoventricular heart-block was present.

CASE 7.—*Arteriosclerosis, cardiac hypertrophy, aortic disease, myocarditis, cardiac failure and death.* (Electrocardiograms, Fig. 4.)

Patient.—W. S., a coal-porter, aged 52, was admitted to the hospital Feb. 24, 1912, complaining of abdominal pain, shortness of breath and sleeplessness.

Family History.—Negative.

Personal History.—The patient gives no history of any past illness. Syphilis and rheumatic fever are both denied. For many years he has drunk six pints of beer daily and has used four ounces of tobacco a week. His work has been heavy.

Present Illness.—The patient states that his present illness began about a month ago (Jan. 24, 1912). At this time he first noticed shortness of breath on any slight exertion. This was soon followed by dull aching pains referred to the upper abdomen, and by sleeplessness. The shortness of breath has gradually increased, a troublesome cough has developed and latterly he has complained of feeling sick on rising in the morning.

Condition on Admission.—The patient is pale and orthopneic. There is no cyanosis. The teeth are carious. The temperature is normal. There is neither dropsy nor ascites. The respirations are 36 to the minute. The urine is normal. The pulse is regular, 90 to the minute, of the water-hammer type. Capillary pulsation is present. Pulsation of the carotids is visible to the lobes of the ear. The arteries are thickened. The systolic blood-pressure is 110-140 mm. Hg. The liver is palpable an inch below the costal margin.

Heart.—The point of maximum impulse is in the fifth and sixth interspaces five inches from the midsternal line. The upper limit of cardiac dulness lies at the third rib; to the right one inch and to the left five inches from the midsternal line. At the apex and over the aortic area systolic and early diastolic murmurs are heard; the first sound is inaudible.

Subsequent History.—During a month's stay in the hospital his acute symptoms, with the exception of slight breathlessness on exertion, entirely subsided. The respirations fell to 20 per minute when at rest. Otherwise his condition remains unchanged. He left the hospital but died some time afterward; no necropsy was obtainable.

CASE 8.—*Cardiac hypertrophy, myocarditis, mitral disease and pericarditis.*

Patient.—H. C., a domestic, aged 18, was seen March 16, 1912, complaining of shortness of breath and some pain in the chest, in front. A note made at this time states that the patient had pneumonia at the age of 5; she is also said to have had swelling of the knees and ankles on several occasions, which suggest a rheumatic history. There has been at different times some edema of the legs. Infantilism is conspicuous.

Heart.—The limit of cardiac dulness lies 3½ inches to the right and 6 inches to the left of the midsternal line. There is an early diastolic and systolic thrill at the apex. The first sound at the apex is reduplicated, associated with early diastolic murmurs. A friction-sound is heard over the base of the heart. The aortic and pulmonary sounds are normal.

Subsequent History (June 14, 1913).—The patient is alive, but her condition is much as noted above.

Electrocardiograms.—The *P-R* interval measures 0.29 second. In Lead I, *S'* and *T'* deflections are seen, the whole curve being diphasic and the initial phase (*S'*) long. In Lead II the curve is similar, but a small spike *R'* is added. In Lead III, *R'* opens the complex and is followed by a *T'* deflection; a small *S'* is also present.

CASE 9.—*Myocarditis, aortic regurgitation, cardiac failure and death.*

Patient (Electrocardiograms, Fig. 5).—S. R., a gas-fitter, aged 36, was admitted to the hospital April 9, 1912, complaining of shortness of breath, cough, swelling of the feet and sleeplessness.

Personal History.—There is no history of any past illness. The patient has seen service in South Africa.

Present Illness.—The onset of his present illness is attributed by the patient to an attack of sore throat and bronchitis, which he had four months ago (January, 1912). He was examined at this time and his heart was found to be

affected. Recently the shortness of breath has increased to such an extent that he cannot take the slightest exercise and work is impossible.

Condition on Admission.—The patient is pale but well nourished. There is slight cyanosis. The respirations are hurried, 28 to the minute, a little labored. Some venous engorgement and signs of pulmonary congestion are present. There is edema of the feet and back. The specific gravity of the urine is 1.023, reaction acid; no albumin is present. The liver is palpable three finger-breadths below the costal margin. The pulse is regular, 106 to the minute, of the water-hammer type. Capillary pulsation is present, though slight. The arteries are tortuous.

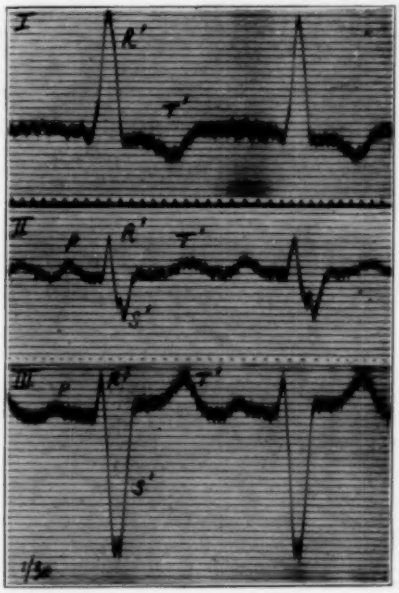

Fig. 5.—Electrocardiograms, Case 9. The *P-R* interval measures 0.17-0.18 second. The *Q-R-S* interval measures 0.1 second to 0.11 second. In Lead I, the initial deflection *R'* is conspicuous as a tall broad summit with slight notching. *T'* is inverted lying in the opposite direction to the summit *R'*. In Lead II, the initial deflections *R'* and *S'* are equally conspicuous. *S'*, however, shows marked notching. *T'* is upright in a direction opposite to the deflection *S'*. In Lead III, the deflection *R'* is present as a short summit, while the depression *S'* is conspicuous as a deep broad deflection with split base. *T'* is upright in a direction opposite to the prominent deflection *S'*. The greatly increased amplitude, the longer duration and the notching of the initial deflections *R'* and *S'* are conspicuous in all leads.

Heart.—The shock of the heart is diffuse and extends well outside the nipple line. The limit of cardiac dulness lies 2 inches to the right and 6¼ inches to the left of the midsternal line. At the apex a systolic and a faint diastolic murmur are heard; over the aortic area to-and-fro murmurs are heard, maximal in intensity at this point; over the pulmonary area the same murmurs, though fainter, are audible. The second sounds are not heard.

Course.—After a month's stay in the hospital the patient's condition improved considerably, and he was discharged at his own request. A few weeks later he was admitted to an infirmary, with an exacerbation of all his symptoms and with aggravated dropsy. He died within a few hours. No necropsy was obtained.

CASE 10.—*Rheumatic carditis, aortic disease, angina pectoris, high blood-pressure, alternation, cardiac failure and death.* (Electrocardiograms, Fig. 6.)

Patient.—R. B., aged 45, came under observation June 6, 1912, complaining of pains in the chest and breathlessness.

Family History.—The patient's father and an uncle died suddenly of "heart disease." A sister has had rheumatic fever. The patient's child, aged 9, was examined and found to have mitral stenosis.

Personal History.—The patient has suffered from rheumatic fever on five occasions, the first attack being at the age of 12 and the last at 30. He became almost deaf at 40. At 43 he had an attack of pleurisy. Syphilis is denied.

Present Illness.—The present illness really began twenty-eight years ago, when the patient was treated at a London hospital for angina pectoris. The pains have been severe of late and radiate over a wide area; they are accompanied by feelings of giddiness, suffocation and anxiety, and are brought on by the slightest exertion. Lately his legs have become swollen and breathlessness has developed.

Condition on Admission.—The patient is pale and poorly nourished. There is a little dropsy of the extremities. Cyanosis and dyspnea are absent. The

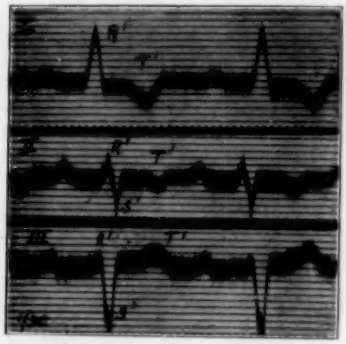

Fig. 6.—Electrocardiograms, Case 10. The *P-R* interval measures 0.21 to 0.23 second. In Lead I the initial deflection *R'* appears as a slightly broadened peak with notching of the side and apex. *T'* is inverted lying in the opposite direction to the summit *R'* In Lead II, the initial deflection *R'* is conspicuously notched. The deflection *S'* is plainly seen. *T'* it will be noted is again inverted, lying in a direction opposite to *R'*. In Lead III, the greatly increased amplitude of the initial deflection *S'* is apparent. *S'* shows marked splitting of its base. *R'* is inconspicuous. *T'* is upright lying in a direction opposite to the prominent initial deflection *S'*. The increase in the *P-R* interval is apparent in all leads

carotids pulsate vigorously and the veins of the neck are abnormally prominent. The liver is not enlarged. The urine contains no albumin. The pulse is of the water-hammer type and occasionally intermits. Capillary pulsation is present. The alteries are thickened. The systolic blood-pressure lies between 180 and 200 mm. Hg.

Heart.—The visible impulse is diffuse. The upper limit of cardiac dulness is at the second rib; the limit of dulness to the right is 1½ inches and to the left 7½ inches from the midsternal line. At the apex the first sound is replaced by a systolic bruit, followed by an early diastolic murmur. At the aortic cartilage a diastolic thrill and murmur are detected; the second sound is absent.

Course.—The patient was under observation for over twelve months, during which period the physical signs and symptoms remained unaltered. With rest the pain disappeared, only to reappear as soon as the patient was about again.

He died suddenly in August, 1912. No post-mortem examination could be obtained.

Electrocardiograms.—The electrocardiographic curves were constantly of the same type, being those associated with deficient conduction of the right branch of the auriculoventricular bundle. In this case extraventricular systoles were recorded appearing in a direction opposite to the prominent initial deflections in all leads, hence the premature beat arose in the opposite ventricle from the aberrant beat, that is, from the right and basal portion of the heart.

CASE 11.—*Arteriosclerosis, myocarditis, aortic disease, cardiac failure and death.* (Electrocardiograms, Fig. 7).

Patient.—C. H., a seaman, aged 47, was admitted to the hospital July 20, 1912, complaining of shortness of breath and swelling of the legs.

Family History.—A brother is said to have had rheumatic fever; otherwise the family history is negative.

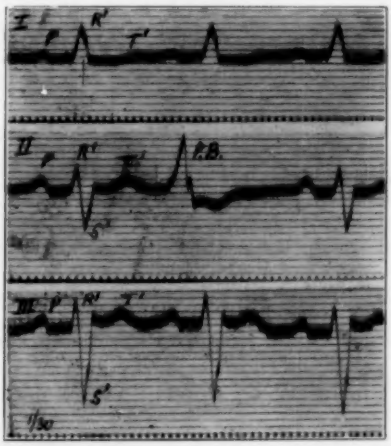

Fig. 7.—Electrocardiograms, Case 11. The *P-R* interval measures 0.2 second. In Lead I the initial deflection *R'* appears as a somewhat broadened summit with marked notching. *T'* is upright in the same direction. In Lead II, the initial deflection *R'* is plain. The deflection *S'* shows an increase in amplitude and conspicuous notching. *T'* is upright lying in a direction opposite to the prominent *S'*. The single premature beat recorded in the figure—*P-B.*—coincides with the auricular summit *P*, which does not appear. The premature beat lies in a direction opposite to the prominent aberrant deflection *S'*. In Lead III, the initial deflection *R'* is present. The deflection *S'* appears as a conspicuous depression of greatly increased amplitude, with slight notching. *T'* is upright in a direction opposite to the prominent deflection *S'*. The premature beats recorded in this lead (none is shown in the figure), were in the same direction as the aberrant curve.

Personal History.—There is no history of any illness, with the single exception of an attack of "bronchitis," which the patient had eighteen months ago. He has never had rheumatic fever. He denies syphilis.

Present Illness.—The patient attributes the onset of his present illness to the "bronchitis" which he had eighteen months ago. This attack was soon followed by shortness of breath, which has increased steadily. During the past weeks his legs and abdomen have become swollen.

Condition on Admission.—The patient is florid. The conjunctivae are tinged with bile. He is cyanotic and very orthopneic. There are signs of fluid in the right chest. There is marked edema of the back, legs and scrotum, and abdomi-

nal ascites is present. The liver is palpable below the umbilicus and pulsating. The respirations are 32 to the minute. The temperature is normal. The specific gravity of the urine 1.012; the normal amount is reduced and albumin is present in excess. The pulse-rate is from 118 to 120 per minute, occasionally intermittent, of the water-hammer type. Capillary pulsation is present. The arteries are thickened. The systolic blood-pressure is low, from 98 to 102 mm. Hg.

Heart.—The point of maximum impulse is neither visible nor palpable. The upper limit of cardiac dulness lies at the fourth interspace, to the right at the right sternal margin and to the left 4½ inches from the midsternal line. There is an apical systolic murmur and a faint first sound. At the aortic area systolic and diastolic murmurs are heard.

Course.—The patient was given infusion of digitalis (2 drams three times daily), but showed no improvement. He died July 27, a week after admission. Necropsy was refused.

Electrocardiograms—In three observations, extending over a period of six weeks, the *P-R* interval measured 0.26, 0.16 and 0.2 second, the last two days before death. The curves were always of the same type, being those seen in association with defective conduction of the right branch of the auriculoventricular bundle. The extra systoles recorded were in a direction opposite to the prominent initial deflection *S'*, of the aberrant curve, in Lead II, but in the same direction as the aberrant curve in Lead III (Fig. 7).

CASE 12.—*Myocarditis and aortic disease. Febrile auriculoventricular heart-block of varying degree.* (Electrocardiograms, Figs. 8 and 9.)

Patient.—W. C., a bookbinder, aged 32, was admitted to the hospital Aug. 16, 1912, complaining of shortness of breath and pains in the chest.

Personal History.—The patient states that he always felt perfectly well until about six years ago. He has never had rheumatic fever. He denies syphilis. He is a heavy smoker, but uses alcohol in moderation.

Present Illness.—The patient dates the onset of his present illness back about six years, when he first began to notice shortness of breath after any unusual exertion. This shortness of breath has increased, and for some months now he has had pain of an aching character over the precordial area. He is unable to lie on the right side at night.

Condition on Admission.—The patient, thin and pale, lies propped up in bed and quite breathless. There is no dropsy or liver enlargement. The urine contains no albumin. The pulse is regular, of the water-hammer type, ranging from 64 to 88 per minute. The arteries are thickened. The systolic blood-pressure varies from 110 to 148 mm. Hg. The temperature is 99 F.

Heart.—The heart's impulse is diffuse in the fourth, fifth and sixth interspaces. The limits of cardiac dulness lie at the third rib above, ¾ of an inch to the right and 5½ inches to the left of the midsternal line. At the apex a short, sharp first sound and a low-pitched presystolic murmur are heard. The second sound is faint and is followed by an early diastolic murmur. At the base a systolic thrill is felt, and rough to-and-fro murmurs are audible, having their maximum intensity at the aortic cartilage. The second sound is not heard.

Course.—The temperature rose to 100.5 F. the day after admission, but fell gradually to normal by August 22. After several weeks rest, his symptoms being considerably improved, he was discharged.

Readmission (May 27, 1913). The patient complains of throbbing pain in the chest, which has increased during the past three weeks.

Condition on Readmission.—There is no cyanosis or dyspnea. The pulse is regular in force and rhythm, of water-hammer type. Capillary pulsation present. The arteries are sclerosed, as noted formerly.

Heart.—The cardiac dulness lies at the third rib above, to the right at the right sternal margin, to the left 5½ inches from the midsternal line. There

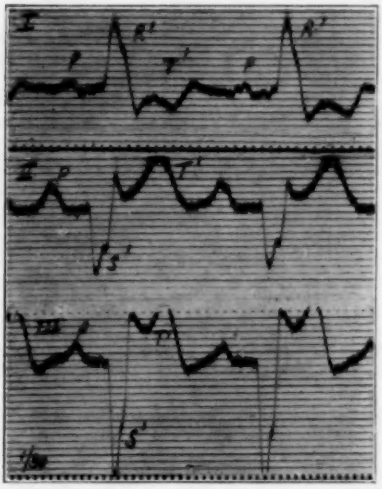

Fig. 8.—Electrocardiograms, Case 12. The *P-R* interval measures 0.18 second. The *Q-R-S* interval measures 0.13 second. In Lead I the initial deflection *R'* appears as a broad, tall peak with conspicuous notching. *T''* is inverted lying in a direction opposite to the prominent deflection *R'*. In Lead II *R'* is wanting, the initial deflection *S'* appears as a deep depression of increased duration as well as amplitude, with conspicuous notching. *T''* is exaggerated, upright, in a direction opposite to the prominent deflection *S'*. In Lead III *S'* is conspicuous as a deflection of greatly increased amplitude with marked notching. *T''* is upright in a direction opposite to that of the conspicuous deflection *S'*. The increase in the *P-R* interval and the great increase in the duration and amplitude of the initial phases of the ventricular complex is apparent in all three leads. The bizarre character of the curves recorded on this date is in striking contrast to the curves taken on the following day.

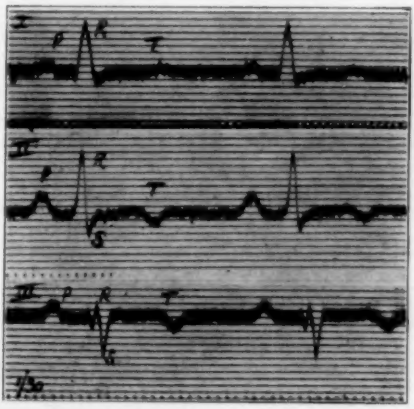

Fig. 9.—Electrocardiograms, Case 12. The *P-R* interval measures 0.18 second. The *Q-R-S* interval measures less than 0.1 second. In Lead I, *R* is present as a peak of short duration and amplitude. *T* is upright, lying in the same direction as the peak *R*. In Lead II, *R* and *S* are present. R appearing as a deflection of short duration. *T* is inverted lying in a direction opposite to the peak *R*. In Lead III the initial deflections *R* and *S* are not exaggerated. *T* is again inverted.

is a systolic as well as a presystolic murmur at the apex, the latter is heard only with every fifth or sixth beat. Over the aortic area there is a double murmur. The specific gravity of the urine is 1.028; a trace of albumin is present.

June 12, 1913: Under rest the patient's condition, so far as subjective symptoms are concerned, has materially improved. His pain has been relieved and he feels much more comfortable. The physical signs remain unchanged.

Electrocardiograms.—This case is of especial interest, in that during the period the patient has been under observation, varying degrees of heart-block have been noted.

A comparison of the electrocardiographic curves taken on the second and third day after his first admission (August, 1912) is most interesting and instructive; the exaggerated, bizarre form recorded August 18 (Fig. 8), the result of defective conduction of the right branch of the auriculoventricular bundle, due to his febrile attack, giving place to the very different curve recorded on the next day (Aug. 19, 1912). On this date the ventricular portions of the curves have entirely altered (Fig. 9). There is no evidence of any defect in conductivity of the right branch of the bundle, but only of a relative preponderance of the left ventricle.

These two figures are most instructive, illustrating as they do the occurrence of a temporary defect in conductivity of the right branch of the auriculoventricular bundle (Fig. 8), and on the subsidence of this acute defect, the physiological electrocardiograms for this patient (Fig. 9) indicative only of a relative left-ventricular hypertrophy. In addition to the very apparent differences in the duration and amplitude of the opening phases, as well as in the direction of the prominent deflections in the various leads of the two figures, a comparison of the direction of the summits T' and T in the corresponding leads is most important. In each of the corresponding leads, of the two figures, it will be noted that where T' points in one direction T points in the other, and that, throughout, T is much less conspicuous than T'.

In Figure 8, T', in accordance with the general rule in these cases, lies in a direction opposite to that of the prominent initial deflections in all leads. In Figure 9 the inversion of T in Lead II is in itself of pathological significance.

Note the absence of notching in the ventricular complex of Figure 9.

These two figures may well serve to emphasize the differences between the curves obtained as a result of a defect in conductivity of the right branch of the auriculoventricular bundle, and those due to a relative hypertrophy of the left ventricle.

CASE 13.—*Rheumatic myocarditis, pleurisy with effusion, displacement of the heart and cardiac failure.* (Electrocardiograms, Fig. 10.)

Patient.—R. A., aged 35, was admitted to the hospital Sept. 11, 1912, complaining of shortness of breath and swelling of the legs.

Family History.—Negative.

Personal History.—At the age of 18 the patient was ill for three months with acute rheumatic fever. Subsequently he had many similar, though less severe attacks, until he was 28. Five months ago he had an acute pleurisy with effusion; was tapped and a large amount of fluid was withdrawn from the left pleura. He has used alcohol in moderation and does not use tobacco.

Present Illness.—During the past seven years the patient has been quite free from any symptoms of acute rheumatic fever, but states that for the past seven months he has suffered from a feeling of exhaustion after slight exertion, and from very marked shortness of breath since his pleurisy five months ago. He further states that, to his knowledge, his heart has been affected for ten years at least.

Condition on Admission.—The patient is thin and poorly developed. He has a bad pyorrhea. He is slightly cyanotic, and dyspnea is marked. On the left side the movements of the chest are limited and tubular breathing is heard at

the apex behind. There is dulness at the base of the left lung and the breath-sounds are weak. Friction-sounds are heard in the axillary region. There is slight edema of both legs. The temperature is normal. The pulse is regular, from 76 to 104 per minute; the arteries are a little thickened; the systolic blood-pressure varies from 98 to 134 mm. Hg.

Heart.—The point of maximum impulse lies in the fourth interspace, 2 inches inside the right nipple-line. There is a diffuse pulsation over the right side of the chest internal to the main impulse. The limits of cardiac dulness lie at the third rib above, and 2½ inches to the right and left of the midsternal line. The first sound is inaudible at the apex, the second sound is accentuated and a coarse systolic murmur is heard. The second sound is reduplicated at both pulmonary and aortic cartilages. A pleuropericardial friction-rub is heard over the left half of the precordium. Roentgenoscopy showed great displacement of the heart to the right, with enlargement, but no trace of fluid could be seen.

Subsequent History.—After a sufficient period of rest in the hospital the patient so far improved as to be able to leave. So far as is known at present (June 14, 1913) he is still living.

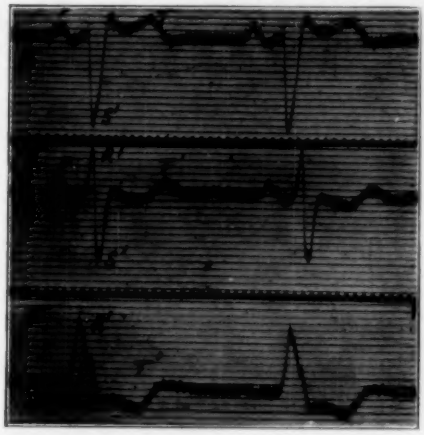

Fig. 10.—Electrocardiograms. Case 13. The *P-R* interval measures 0.13 second. The *Q-R-S* interval measures 0.13 second. In Lead I, *R'* is absent, the initial deflection *S'* appearing as a depression of greatly increased amplitude (apex negativity) with suggestive notching at the extreme base. The summit *T'* is upright, lying in a direction opposite to the prominent deflection *S'*. In Lead II, *R'* is present but the deflection *S'* is of greater amplitude and duration, again with suggestive notching of the base. *T'* is upright in a direction opposite to the prominent deflection *S'*. In Lead III, the initial deflection *R'* is present as a conspicuous summit of increased duration and amplitude, with marked notching. *T'* is again conspicuous, inverted, lying in a direction opposite to the prominent initial deflection *R'*. When compared with all the other accompanying figures, it will be seen that the direction of the prominent initial deflections in Leads I and III are in the opposite direction. It has been shown that in lesions involving the left branch of the auriculoventricular bundle the initial deflection in Lead I is always downward (apex negative) while in Lead III, it is always upward (base negative).

This case is of especial interest in that it is the single instance, in the series, of involvement of the left branch of the auriculoventricular bundle.

CASE 14.—*Arteriosclerosis, cardiac hypertrophy, myocarditis, alternation, cardiac failure and death.*

Patient.—W. P., a waterside laborer, aged 62, was admitted to the hospital Nov. 1, 1912, complaining of faintness and shortness of breath.

Personal History.—With the exception of an attack of pleurisy twelve years ago, there is no history of any acute illness. The patient has had repeated attacks of tonsillitis to which he has paid little attention. He also states that he has suffered from so-called "muscular rheumatism" for many years, but that he had not had acute rheumatic fever. A sister is said to have had a history of vague "rheumatic" pains. He denies syphilis. He has used but little alcohol and smokes in moderation.

Present Illness.—For the past eighteen years the patient has felt less fit for work than previously, and from time to time has experienced shortness of breath. Eight years ago while carrying heavy wheat-sacks he had an attack of faintness and was forced to stop work. He noticed at this time that his scrotum was a little swollen and that it increased in size during the next two days. He was treated at a hospital, improved and went back to work. A year later he had a similar attack and was again incapacitated. He has not been well since this attack in 1906, and has been treated at several institutions recently for an aggravation of the same symptoms and faintness.

Condition on Admission.—The patient is pale and spare. The conjunctivae are bile stained; there is a little cyanosis of the mucous membranes. He has hurried and irregular breathing. Dropsy of the legs, genitalia and scrotal region is present. The urine is acid, specific gravity 1.018, and a trace of albumin is present. The liver is enlarged and there is some ascites. There is dulness at the left base of the chest with weak breath-sounds; faint râles are heard at both bases. The temperature is normal.

Heart.—The heart's dulness extends 2 inches to the right and 6½ inches to the left of the midsternal line. There are no murmurs. The heart-beats are either regular at 80 or 90 per minute, when cantering rhythm is heard; or the beats are in couples, when three sounds accompany each beat of the couple. When the coupled beats are present the pulse-rate is halved. The arteries are thickened; the systolic blood-pressure is 120 mm. Hg. Venous pulsation in the veins is conspicuous and these vessels are enlarged.

Course.—After a prolonged stay in the hospital, the dropsy and many of the signs of engorgement were relieved, and the breathing was much freer. The patient died Feb. 13, 1913, shortly after leaving the hospital.

Electrocardiograms.—The P-R interval could not be determined owing to the presence of auricular fibrillation. The ventricular complexes were similar to those shown in Figure 3. The premature beats recorded were in all leads — I, II and III — in a direction opposite to the aberrant beat, and hence arose from the right and basal portion of the ventricle.

CASE 15.—*Myocarditis in gouty subject, anginal symptoms, syncopal attacks, extrasystoles and alteration.* (Electrocardiograms, Fig. 11).

Patient.—J. W., a man, aged 68, was first seen Nov. 6, 1912, complaining of attacks of faintness and occasional loss of consciousness.

Personal History.—For many years the patient has been subject to acute attacks of gout. For a few years he has had occasional attacks of faintness and throbbing in the chest and a feeling of great distress; these symptoms coming on always after exertion. In some of the attacks he has lost consciousness for a few seconds. These attacks have not been frequent, and as a rule have been isolated, but on one occasion five attacks occurred one after another. The feeling of distress is relieved by nitroglycerin.

Condition on Admission.—The patient is heavily built and shows little or no dyspnea or cyanosis. The urine contains no albumin and there are no signs of dropsy. The pulse intermits occasionally, the rate is 78 to the minute. The systolic blood-presure is 160 mm. Hg.

Heart.—The impulse is diffuse, there is an extension of dulness towards the left side. No murmurs are heard, but the first sound is reduplicated at the apex and base.

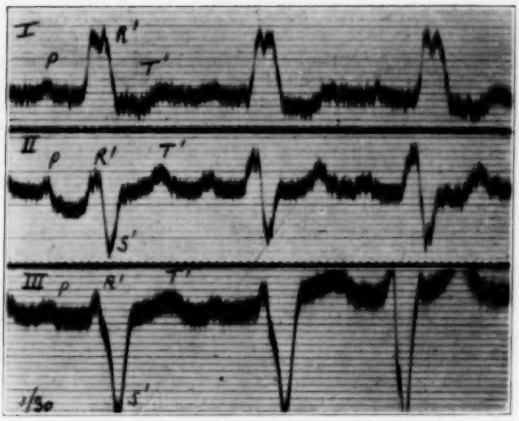

Fig. 11.—Electrocardiograms, Case 15. The *P-R* interval measures 0.2 second. The *Q-R-S* interval measures 0.16 second. In Lead I, the initial deflection *R'* appears as a broad wide summit, with conspicuously split top. The final deflection *T'* is inverted lying in a direction opposite to the deflection *R'*. In Lead II, *R'* is present as a short summit with marked notching, the deflection *S'* is conspicuous as a deep depression with notching, the final deflection *T'* is upright lying in a direction opposite to the prominent deflection *S'*. In Lead III, *R'* is inconspicuous, *S'* appears as a deep depression of exaggerated amplitude and duration, with marked notching. The final deflection *T'* is again upright, lying in a direction opposite to the prominent deflection *S'*. The increase of the *P-R* interval and the increase in the duration and amplitude of the early phases of the complex is apparent in all leads. The single premature beat recorded in Lead III is in the same direction as the prominent deflection *S'*.

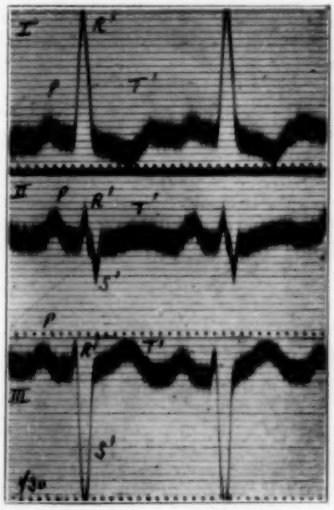

Fig. 12.—Electrocardiograms, Case 16. The *P-R* interval measures 0.14 second. The *Q-R-S* interval is not increased, measuring 0.08 second. In Lead I, the initial deflection *R'* is conspicuous as a tall peak of greatly increased amplitude. The final deflection *T'* is inverted lying in a direction opposite to the prominent summit *R'*. There is no notching. In Lead II, the initial deflection *R'* is present and equally as conspicuous as the deflection *S'*. The final deflection *T'* is upright. In Lead III, the initial deflection *R'* is inconspicuous, the deflection *S'* appearing as a deep depression of greatly increased amplitude. The final deflection *T'* is upright in a direction opposite to the conspicuous deflection *S'*. The increased amplitude of the initial deflections in Leads I and III is apparent, as is the direction of the final deflection *T'* in relation to *R'* and *S'*.

Graphic Records.—The intermissions are the result of premature contractions arising in the ventricle. They are either isolated or occur in short groups. The premature beats are followed by alternation of the pulse.

CASE 16.—*Myocarditis, alternation, Cheyne-Stokes breathing, high blood-pressure and cardiac failure.* (Electrocardiograms, Fig. 12.)

Patient.—D. E., a physician, aged 54, was seen Nov. 16, 1912, complaining of breathlessness on exertion.

Personal History.—The patient had an attack of erysipelas when 15. Otherwise, with the single exception of an attack of pleurisy some years ago, his history is negative. He has been moderate in his use of alcohol, but has always eaten very heartily. His life has been very active.

Present Illness.—For three months past he has suffered from breathlessness, which has been increasing steadily. At present it is brought on by even slight exertion. There has been no pain, soreness of the chest or palpitation.

Condition on Admission.—The patient is heavily built, florid and a little cyanosed. He is of a nervous temperament. There is slight dropsy of the legs, but no enlargement of the liver. He has a tendency to Cheyne-Stokes breathing. The urine contains no albumin.

Heart.—The apex-beat cannot be found. The area of the heart's dulness is increased to the right and left. At the apex, gallop rhythm is heard. There are no murmurs. At the base the sounds are normal. The pulse intermits occasionally; the arteries are much thickened; the systolic blood-pressure is 210 mm. Hg.

Subsequent History.—The patient went home and died in August, 1913,

CASE 17.—*Rheumatic myocarditis, cardiac hypertrophy, aortic disease, ventricular extrasystoles and cardiac failure.* (Electrocardiograms, Fig. 13.)

Patient.—W. O., a hotel porter, aged 27, was admitted to the hospital Feb. 4, 1913, complaining of pain in the chest, ankles and knees.

Family History.—Negative.

Personal History.—The patient had chorea at 11, and rheumatic fever at 17. Following the rheumatic fever, ten years ago, his heart was affected, and he has had shortness of breath ever since.

Present Illness.—The patient refers the commencement of his present acute symptoms to the sudden onset, about ten days ago (January 26), of a sharp and severe pain in the chest, in the region of the heart, which is aggravated by breathing or by coughing. Three days later his ankles became swollen, red and tender, and subsequently his knees also. The patient has been short of breath for the past ten years, but this has been much worse during the past week.

Condition on Admission.—The patient's temperature is 101 F. Both knees and ankles are swollen, but not red or tender; he is sweating profusely. The pulse is typically water-hammer in character, showing occasional extrasystoles. Capillary pulsation is plainly visible. There is a marked pulsation of the vessels in the neck. The arteries are not thickend. No rheumatic nodules are present.

Heart.—There is pronounced heaving of the entire left side of the chest, and a distinct shock palpable in the epigastrium. The upper limit of cardiac dulness lies at the third rib, to the right 1½ inches and to the left 6½ inches from the midsternal line. The apex is well outside the left nipple line in the fifth interspace. The first and second sounds are reduplicated; the first over a wide area, heard 3 inches to the right in the fifth interspace. Over the apex there is a systolic murmur. Over the aortic area there is a systolic and a diastolic murmur, the latter being conducted over the entire heart. On admission there were signs of pleurisy with effusion at both bases, and a general bronchitis. The liver was palpable 1½ inches below the costal margin, no pulsation was present.

Course.—By February 14 the temperature had fallen to normal, the swelling of the joints had subsided, the signs of effusion in the chest had wholly disappeared, and the dyspnea was greatly relieved. He was later discharged from the hospital.

Subsequent History (June 18, 1913.)—The patient is alive and in fair condition.

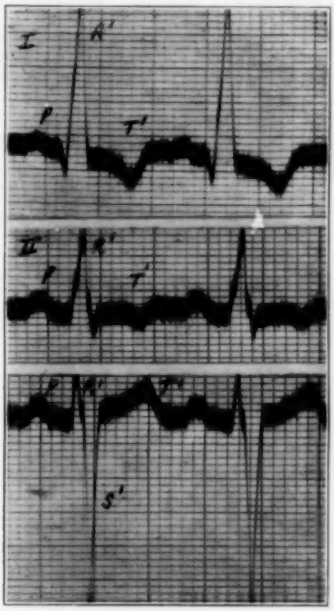

Fig. 13.—Electrocardiograms, Case 17. The *P-R* interval measures 0.17 second. The *Q-R-S* interval measures 0.08 second. In Lead I, the initial deflection *R'* is conspicuous as a tall peak of greatly increased amplitude. The final deflection *T'* is inverted, lying in the opposite direction. In Lead II, the initial deflection *R'* is conspicuous as a summit of exaggerated amplitude with marked notching. *S'* is inconspicuous. The final deflection *T'* is again inverted, lying in a direction opposite to the prominent deflection *R'*. In Lead III, the deflection *R'* is present as a short notched peak. The deflection *S'* is conspicuous as a depression of enormously exaggerated amplitude. The final deflection *T'* is upright in a direction opposite to the conspicuous deflection *S'*. The greatly increased amplitude of the initial deflections is apparent at a glance, as is the direction of the conspicuous final deflection *T'* in relation to the prominent deflections *R'* and *S'*.

This case is of especial interest in view of the varying degrees of heart-block noted, the *P-R* interval varying between 0.28 and 0.17 second in curves taken on different occasions.

CASE 18.—*Arteriosclerosis, cardiac hypertrophy, aortic disease, acute pleurisy, cardiac failure and death.* (Electrocardiograms, Fig. 14.)

Patient.—G. C., a milk-carrier, aged 47, was admitted to the hospital Feb. 7, 1913, complaining of shortness of breath, sleeplessness and pain in the chest.

Family History.—Negative. No history of rheumatic fever in the family.

Personal History.—There is a history of syphilis twenty-four years ago, at the age of 23, and of a severe attack of typhoid fever twelve years ago. There is no history of rheumatic fever or of any other illness. The patient has used alcohol and tobacco in moderation.

Present Illness.—About 18 months ago (August, 1911), the patient first began to experience attacks of pain, stabbing in character, starting in the front of the chest over the lower end of the sternum and passing through to the back between the shoulder blades. These attacks came on chiefly at night, and lasted from half an hour to two hours. The pain was often worse just under the left shoulder blade, and the cessation of the pain was frequently followed by paroxysms of breathlessness. A week after the onset of these symptoms he noticed that his feet began to swell, and a few days later he was obliged to give up work. During the past six weeks he has had frequent attacks of distressing shortness of breath associated with this pain in the chest, coming on even though lying quietly in bed; he has also had a troublesome cough unaccompanied with any expectoration.

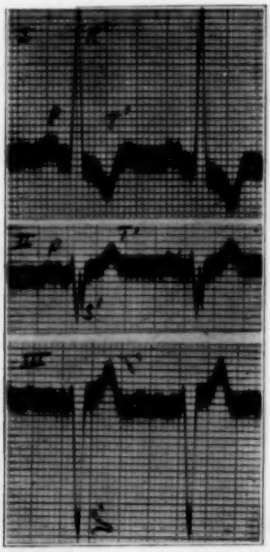

Fig. 14.—Electrocardiograms, Case 18. The *P-R* interval measures 0.2 second. In Lead I, the initial deflection *R'* is conspicuous as a tall peak of greatly increased amplitude. The final deflection *T'* is conspicuous and inverted, lying in a direction opposite to the prominent summit *R'*. In Lead II, the deflection *R'* is absent, the initial deflection *S'* is conspicuous as a markedly split depression. The final deflection *T'* is upright lying in a direction opposite to the prominent deflection *S'*. In Lead III, the initial deflection *S'* is conspicuous as a deep depression of greatly increased amplitude. The final deflection *T'* is upright, lying in a direction opposite to the prominent deflection *S'*. The diphasic character of the complex in Leads I and III, the greatly increased amplitude of the initial deflections, and the direction of the conspicuous final deflection *T'*, in relation to the prominent initial deflections, are apparent.

Condition on Admission.—The patient, a well-developed man, is lying propped up in bed, quite dyspneic. The mucous membranes are pale. There is marked pulsation of the vessels in the neck. The pulse is full and regular in force and rhythm, not compressible, typically water-hammer in character when the arm is raised. The vessel-wall is thickened.

Heart.—The cardiac impulse is visible in the fifth, sixth and seventh interspaces. In the fifth interspace in the anterior axillary line it is heaving. There is a systolic retraction 1 inch below the left nipple. The upper limit of cardiac dulness lies at the third interspace, to the right 1¾ inches and to the left 6½

inches from the midsternal line. At the apex a soft double murmur can be heard over a wide area. Both of these murmurs can be followed up the left border of the sternum. At the aortic cartilage the first sound is obscured by a harsh systolic murmur transmitted upward into the vessels of the neck. The second sound is distant and followed by an early diastolic murmur transmitted downward. The first sound is not heard anywhere.

Respiratory System.—Normal but for a few fine crepitations at both bases posteriorly.

Liver.—The liver is palpable a hand's breath below the costal margin. The spleen is not palpable. There is marked edema of both legs. The specific gravity of the urine is 1.006; a trace of albumin is present but no sugar. No casts seen.

During subsequent observation up to the time of death, April 10, the patient's general condition varied but little from day to day. By February 25 the edema of the legs had disappeared, only to return again later. The pain in the chest was persistent, coming on in paroxysms which left him very dyspneic and greatly depressed.

The cardiac signs remained practically unchanged, though by March 10 the diastolic murmur at the base had wholly obscured the second sound.

About April 1, the pain in the chest became more pronounced, the attacks came on more often and persisted longer. April 5 the patient complained of great pain in the lower right side, and a friction-rub was discovered, heard over the four lower interspaces on the right side behind. April 6 there was well-marked dulness over this area, associated with weak respiratory sounds and decreased vocal resonance and fremitus. On this day a small amount of bright red blood was coughed up. This condition persisted and April 8 a friction-rub was audible over the fifth interspace on the left side anteriorly. On the morning of April 10 a well-marked pericardial friction-rub was heard. There was dulness below the angle of the right scapula, over which area the breath-sounds were weak and the resonance increased. The patient died suddenly during the morning. The temperature just before death was 98 F., and at no time since admission had it been above 99 F.

The systolic blood-pressure was 190 mm. Hg on admission, and had ranged between this figure and 140 mm. Hg, the mean systolic pressure being about 140 mm. Hg. The specific gravity of the urine ranged from 1.006 to 1.028. A trace of albumin was constantly present. No casts were seen.

CASE 19.—*Arteriosclerosis, high blood-pressure, cardiac hypertrophy, myocarditis, mitral disease, nephritis and cardiac failure.* (Electrocardiograms, Fig. 15.)

Patient.—W. J., a coach-builder, aged 42, was admitted to the hospital Feb. 10, 1913, complaining of shortness of breath.

Family History.—Father died of pneumonia, aged 52. Mother died at 47 of a "rheumatic heart."

Personal History.—There is no history of chorea or rheumatic fever. He contracted syphilis in 1889, for which he was treated with mercury over a period of eighteen months. During the winter of 1911 to '12 he had an attack of asthma. He has used alcohol in moderation, tobacco to the extent of 2 ounces a week.

Present Illness.—The patient attributes the onset of his present acute symptoms to the attack of asthma, which he had a year ago. Soon after this illness he began to suffer from shortness of breath and attacks of palpitation, which of late have grown more frequent and have been very distressing. Eight months ago he began to cough and from this time has had a persistent cough accompanied by a frothy expectoration, at times slightly streaked with blood. For the past two months he has noticed that he has voided less urine than usual. He has slept poorly.

Condition on Admission.—The patient is a stout man who looks older than he is. As he lies propped up in bed there is slight dyspnea. The respirations are 28. The chest moves but little and there is some impairment of resonance over the left base posteriorly. Over this area the respiratory sounds are weak. Sonorous and sibilant râles are heard over the entire chest. There is no clubbing of the fingers. The pulse is regular in force and rhythm, 100 per minute. The systolic blood-pressure is 186 mm. Hg. The liver is palpable 3 inches below the costal margin. There is slight edema of the feet. The temperature is normal. The specific gravity of the urine, on admission, was 1.030, with albumin present in excess and many hyaline and granular casts. The Wassermann reaction is positive.

Heart.—The apex-beat is visible and palpable in the sixth interspace, 6 inches from the midsternal line. The upper limit of cardiac dulness lies at the third interspace, to the right 1½ inches, and to the left 6 inches from the midsternal line. At the apex the first sound is loud and booming, accompanied by a soft systolic murmur; the second sound is short and valvular in character. The aortic second sound is accentuated, no murmurs are heard over the aortic area.

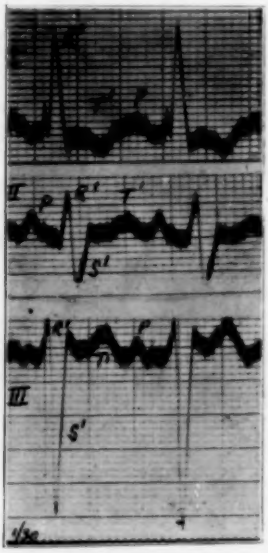

Fig. 15.—Electrocardiograms, Case 19. The *P-R* interval measures 0.2 second. In Lead I, the initial deflection *R'* is conspicuous as a tall peak of increased amplitude, with conspicuous notching. The final deflection *T'* is inverted, lying in the direction opposite to the deflection *R'*. In Lead II, *R'* and *S'* are both present and both show notching. The deflection *S'* is slightly the more conspicuous. The final deflection *T'* is upright, lying in a direction opposite to *S'*. In Lead III, *R'* is present, but the deflection *S'* is conspicuous as a deep depression of greatly increased amplitude. The final deflection *T'* is upright, in a direction opposite to the prominent *S'*. The increased amplitude of the initial deflections, the increased duration of the opening phases—the *Q-R-S* interval measures 0.12 second—and the direction of the final deflection *T'*, in relation to *R'* and *S'*, are apparent.

Subsequent History.—After a few weeks rest the acute symptoms were much relieved and the patient left the hospital. He was readmitted at a later date and died in the hospital. The *P-R* interval measures 0.18 and 0.2 second (two observations).

CASE 20.—*Myocarditis, cardiac hypertrophy, aortic disease, cardiac failure and death.* (Electrocardiograms, Figs. 16 and 17.)

Patient.—D. H., a laborer, aged 38, was admitted to the hospital Feb. 25, 1913, complaining of shortness of breath and pain in the chest in the region of the heart.

Family History.—Mother is said to have died of "heart disease." No history of rheumatic fever in the family.

Personal History.—There is no history of any acute illness. The patient has never had chorea or rheumatic fever. He had syphilis fourteen years ago. He has used alcohol in moderation.

Present Illness.—The patient states that his present illness began five months ago, when he first noticed that he became short of breath after any slight exertion. This was soon followed by a sense of fulness and oppression in the chest. During the past month he has had attacks of pain in the chest referred chiefly to the region of the second and third interspaces on the left side. These attacks have latterly become more frequent, and at present the pain is quite constant. There has been no cough.

Condition on Admission.—The patient is well-built, slightly cyanotic and quite dyspneic. The pulse is regular in force and rhythm, typically water-hammer in character. Capillary pulsation is present. The liver is palpable below the costal margin.

Heart.—The point of maximum impulse is diffuse in the sixth interspace almost at the anterior axillary line. There is a palpable systolic thrill at the apex. The upper limit of cardiac dulness lies at the third rib, to the right 1¾ inches and to the left 6 inches from the midsternal line. Over the aortic area there is a double murmur, and passing upward a short systolic murmur is heard over the vessels in the neck. Passing downward the diastolic murmur becomes louder, being most intense over the third and fourth interspaces. As one passes outward toward the apex a rough rumbling murmur is heard just before and associated with doubling of the first sound, suggesting a Flint murmur.

Course.—After several weeks the patient's general condition so far improved that he was able to leave the hospital, being at the time of his discharge practically free from pain, though still dyspneic upon exertion. The area of cardiac dulness had slightly diminished, but otherwise the physical signs remained unchanged, though at this time the rumbling murmur heard at the apex was not clearly associated with reduplication of the first sound.

Readmission (May 15, 1913).—The patient was readmitted to-day, all his old symptoms being more pronounced. While he was in the out-patient department, the following note was made: Breathlessness more marked, slight cyanosis and excessive urobilin staining. There is a slight effusion at the base of the left lung. The liver is palpable at the level of the umbilicus.

Heart.—The apex-beat is diffuse in the sixth interspace. The limits of cardiac dulness are to-day at the third rib above, 2 inches to the right and 6½ inches to the left of the midsternal line. There is a systolic thrill at the apex and a systolic and a full diastolic rumble are heard over this area. Over the aortic and pulmonary areas there is a double murmur.

Course.—The patient's general condition grew gradually worse and he died in June, 1913.

Electrocardiograms.—This case is of more than usual interest in view of the widely varying degrees of heart-block recorded. The *P-R* interval ranged from 0.21 to 0.3 second. A comparison of the electrocardiographic curves recorded February 27 (Fig. 16) and again May 5, 1913, (Fig. 17), is most interesting and instructive.

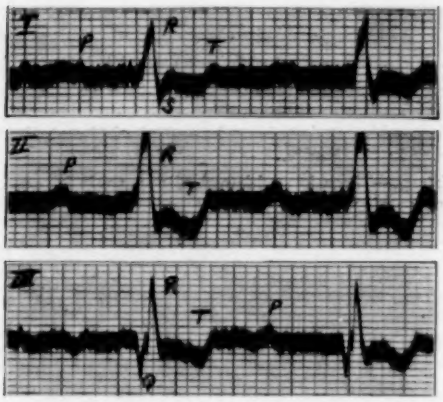

Fig. 16.—Electrocardiograms, Case 20. Before the development of the lesion involving the right branch of the auriculoventricular bundle. The *P-R* interval measures 0.3 second. In Lead I, *Q* is absent, *R* is prominent and already shows notching. *S* is inconspicuous. The final deflection *T* is upright. In Lead II, the initial deflection *R* is more prominent than in the first lead. The final deflection *T* is inverted. In Lead III, *Q* is conspicuous, *R* is prominent, and the final deflection *T* is again inverted. The increased *P-R* interval, the suggestive notching of the prominent deflection *R*, and the inversion of *T* are the sole signs of a disturbed mechanism, the curve is essentially that of cardiac hypertrophy.

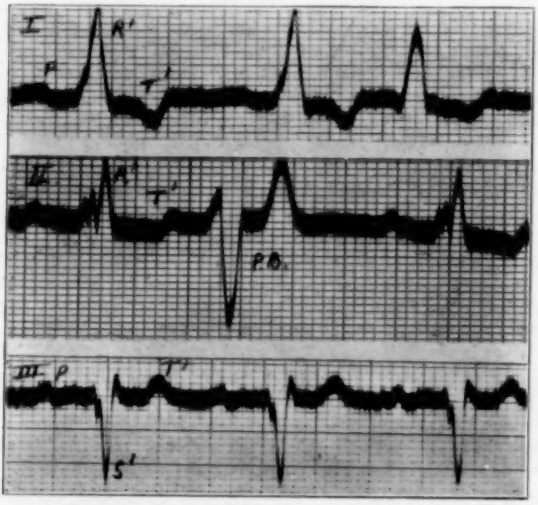

Fig. 17.—Electrocardiograms, Case 20. The *P-R* interval measures 0.22 second. The *Q-R-S* interval measures 0.12 second. In Lead I, the initial deflection *R'* appears as a tall wide peak, of greatly increased amplitude, with marked notching. The final deflection *T'* is inverted. The premature beat lies in the same direction as *R'*. In Lead II, the initial deflection *R'* appears as a conspicuously split summit. The final deflection *T'* is upright. The premature beat lies in a direction opposite to the conspicuous initial deflection *R'*. In Lead III, *R'* is absent, the conspicuous initial deflection *S'* appears as a deep depression, of increased amplitude, and with marked notching. The premature beats, it is seen, are in the same direction as the prominent initial deflection *R'* in Lead I, and in the opposite direction in Lead II.

These two figures illustrate again, in a striking way, the difference between the electrocardiogram obtained as a result of simple cardiac hypertrophy, and that due to defective conductivity.

The earlier physiological electrocardiogram for this patient, obtained in the light of a full knowledge of the degree of cardiac hypertrophy present, could not be confused with the aberrant complex obtained later, after the development of the lesion involving the conduction of the right branch of the bundle.

CASE 21.—*Myocarditis, arteriosclerosis, aortic disease and cardiac failure.* (Electrocardiograms, Fig. 18.)

Patient.—J. M., a porter, aged 39, was first seen June 5, 1913, complaining of palpitation of the heart and shortness of breath.

Family History.—Negative.

Personal History.—There is no history of any acute illness. The patient has not had rheumatic fever. There is a history of syphilis fourteen years ago, for which he was under treatment nine months. He uses alcohol and tobacco in moderation.

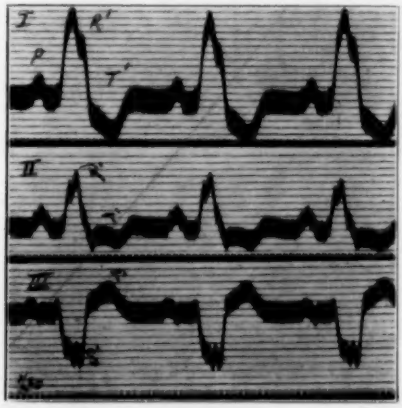

Fig. 18.—Electrocardiograms, Case 21. The *P-R* interval measures 0.17 second. The *Q-R-S* interval measures 0.2 second. In Lead I, the initial deflection *R'* is conspicuous as a tall, broad summit with marked notching. The final deflection *T'* is conspicuous and inverted, lying in a direction opposite to the summit *R'*. In Lead II, the initial deflection *R'* is again conspicuous as a summit of increased height and duration, with marked notching. The final deflection *T'* is inverted, lying in a direction opposite to the prominent summit *R'*. In Lead III, the initial deflection *S'* is conspicuous as a deep, broad depression with marked notching. The final deflection *T'* is upright in a direction opposite to the conspicuous *S'*. The increased amplitude of the initial deflections, the increased duration of the opening phases, the direction of the final deflection *T'* in relation to the prominent initial deflections *R'* and *S'*, the conspicuous notching, and the diphasic character of the complex are apparent.

Present Illness.—The patient states that for the past seven years he has had palpitation of the heart on slight exertion. Beginning in August, 1912, he first noticed that he quickly became very short of breath after any unusual effort. He has had occasional pains in the right shoulder, but nothing that could suggest acute rheumatic fever. Under medical advice he has been doing little work for the past three months, and states that his general condition has improved considerably.

Condition on Admission.—The patient is thin and rather poorly nourished. He is slightly cyanotic and quite breathless on exertion. The pulse is regular in force and rhythm, except for an occasional extrasystole. The arteries are moderately thickened. The systolic blood-pressure is 120 mm. Hg.

Heart.—The point of maximum impulse is in the fifth and sixth interspaces. The limits of cardiac dulness lie at the third interspace above, and 2½ and 5 inches to the right and left of the midsternal line, respectively. There is a well-marked shock over the left side of the cardiac area and considerable pulsation in the second right interspace. There is a systolic thrill at the base and a pronounced diastolic murmur at the aortic cartilage. There is a suggestive Flint murmur at the apex. The liver is just palpable at the costal margin. The Wassermann reaction is positive.

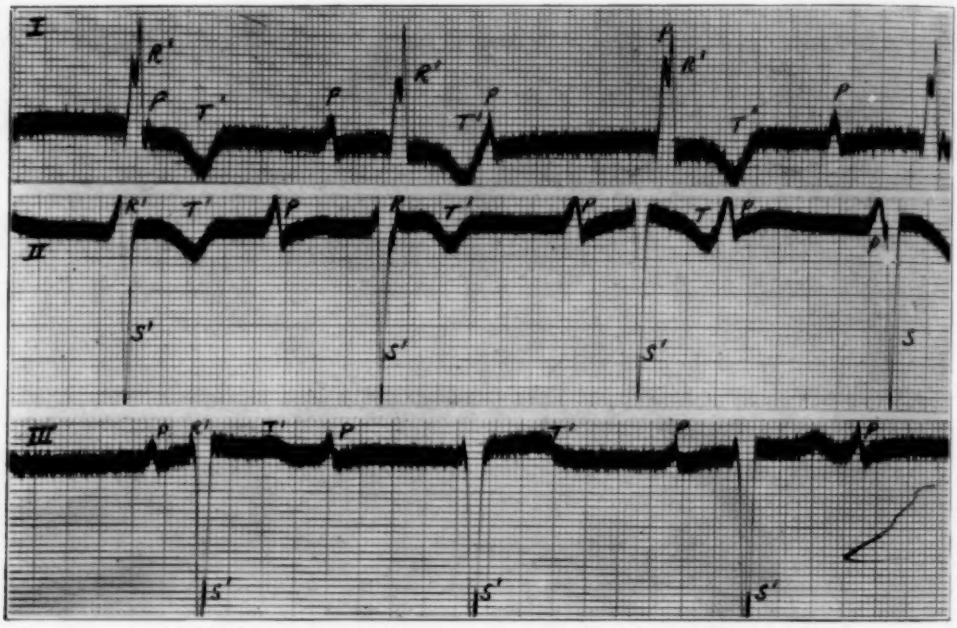

Fig. 19.—Electrocardiograms, Case 22. Note the complete independence of the auricular and ventricular rhythms. The ventricular rate is 39, the auricular rate approximately 60. In Lead I, the initial deflection *R'* is conspicuous as a tall summit, with marked notching of increased amplitude and of slightly increased duration. The final deflection *T'* is conspicuous and inverted, lying in a direction opposite to the prominent summit *R'*. The auricular summit *P* falls just at the conclusion of the deflection *R'* in the first complex of the figure, and obscures the diphasic character of the complex as seen in the second and third ventricular deflections. In Lead II, the initial deflection *R'* is present but not prominent. The deflection *S'* is conspicuous as a deep depression of greatly increased amplitude. The final deflection *T'* is inverted, lying in the same direction as the prominent deflection *S'*. In Lead III, *R'* is just seen. The initial deflection *S'* is again conspicuous as a deep depression of greatly increased amplitude, with notching near the base. The final deflection *T'* is upright in a direction opposite to *S'*. The *Q-R-S* interval exceeds 0.1 second in all three leads. The premature beats recorded in this case were in a direction opposite to the aberrant deflections in Leads I and III, and hence arose from the right and basal portion of the ventricle.

Case 22.—*Congenital pulmonary stenosis, cardiac hypertrophy and complete auriculoventricular heart-block.* (Electrocardiograms, Fig. 19).

Patient.—E. N., aged 27, a domestic, was first seen March 12, 1913, complaining of shortness of breath, of a "winter cough" for as far back as she can remember, and latterly of pain in the chest about the heart.

Family History.—Negative.

Personal History.—Apart from the statement, given above, of shortness of breath, and of the history of a "winter cough" for many years, there is absolutely no other history of any acute illness. The patient has never had scarlet fever, chorea, rheumatic fever or tonsillitis. Careful questioning fails to elicit any history at all suspicious of a syphilitic infection. She states that, as a young girl at school, she frequently used to "feel faint," but that she never fell down or lost consciousness. She has had no such sensations of faintness now for many years. She has had no palpitation or sense of disturbance as of sudden stoppage of the heart. There is no history of any giddiness or dizziness in recent years.

Present Illness.—The patient has been in domestic service for the past seven years, doing hard, general work, which often involved heavy lifting. She first began to notice more aggravated shortness of breath about a month ago (February 12), and found that she could not do her work as usual without quickly becoming very breathless and fagged out. Three weeks ago (February 19) she first complained of pain in the chest, in the region of the heart, of sudden onset, quite sharp, and described as jumping in character. This pain is not constant, but comes on quite frequently three to eight, or more times a day. The pain has never been referred to the left shoulder or down the left arm.

Condition on Admission.—The patient is slightly built and rather poorly nourished. There is considerable cyanosis of the lips and hands, but no clubbing of the fingers. The pulse is of small volume, regular in force and rhythm, 39 to the minute.

Heart.—The point of maximum impulse lies in the fifth interspace in the nipple line, but there is also a pulsation in the anterior axillary line. The upper limit of cardiac dulness lies at the third interspace, to the right 1¾ inches, and to the left 5¼ inches from the midsternal line. There is a pronounced shock over the precordium. There is a well-marked thrill palpable over the upper precordium, and heard at its maximum over the second left cartilage. At the apex the first sound is loud, followed by a soft systolic murmur. The second sound is reduplicated. Over the aortic area there is a faint systolic murmur. The respiratory rhythm is almost synchronous with the ventricular rate, but tracings show that this is not constant.

Course.—The patient has been seen quite regularly, at weekly intervals. Her general condition has improved. By May 19 she was quite free from any attacks of pain, and the extreme breathlessness, for which she first sought relief, had been greatly relieved. May 26 she felt so much better that she asked to be allowed to return to work.

Electrocardiograms.—This case is of more than unusual interest because of the presence of complete auriculoventricular heart-block together with a lesion involving the conductivity of the right branch of the auriculoventricular bundle. It is the single instance of complete auriculoventricular heart-block in our series, and illustrates in an incomparable way, the association of lesions involving the main stem and one of its divisions.

CONCLUSIONS

1. In the presence of lesions involving the conductivity of the branches of the auriculoventricular bundle, a distinct and characteristic type of electrocardiogram is obtained. To the ventricular contractions on which these depend the term *aberrant* may be applied.

2. This type of electrocardiogram is frequently associated with aortic valve disease; it is distinct from that of hypertrophy predominant in one ventricle.

3. Bundle-branch defects are usually accompanied by defects in the main trunk, suggesting that the junctional tissues as a whole have a special pathology.

4. The evidence in our possession is too slight to admit of any definite conclusions as to the relation between lesions of the bundle branches and the occurrence of extrasystoles.

5. The electrocardiographic pictures of aberrant beats are probably of considerable prognostic importance.

I wish to express my indebtedness to Dr. Thomas Lewis, of London, for his kindness in placing his notes and records at my disposal; for many valuable suggestions and the courteous aid received from him in the preparation of this report.

1872 East Ninety-Seventh Street.